Comparative geographic variation of selected southern African gerbils

by

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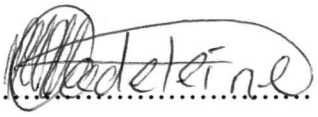
Signature: 

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Abstract

The aims of this study were to describe and compare the geographic variation of *D. auricularis* and *G. paeba*, and determine whether the four recognised subspecies of the latter species are validusing traditional morphometrics and molecular data based on partial sequences of the mitochondrial cytochrome b (cyt *b*) gene.The traditional morphometric analyses were based on 12 cranial variables taken from 89 specimens from 54 localities for*D.auricularis* and 48 *G. paeba* specimens from 25 localities. Variables from both males and females were combined since univariate and multivariate analyses revealed there was no sexual dimorphism in the two species(Wilks' lambda, Λ = 0,942; p = 0.78 for *D. auricularis* and Λ = 0, 81; p = 0.82 for *G. paeba*). Univariate analysis revealed significant age variation and only age class II and IIIwere used for both species(for *D. auricularis,*Λ = 0,713; p = 0.035 and for *G. paeba,* Λ = 0, 748; p = 0.04). Multivariate analyses indicated that there was no intra-population variation which confirms that *D. auricularis* is a monotypic species. However, multivariate results for *G.paeba*suggest that two main operational taxonomic units exist.Variables differentiating these operational taxonomic unitsare mandibular length and rostral height.Overall, morphometricsresults suggest that four subspecies of *G. paeba* can be reduced to two subspecies. This includes *G. p. paeba* which has the widest distribution and consists of specimens from the South West Arid zone and*G. p. coombsi* comprises specimens from Southern Savanna Woodland.

The molecular aspect of this project was also based onmuseum material, albeit from a reduced sample size: *D. auricularis* (N= 41 from 25 localities) and *G. paeba* (N=26 from 12 localities). A fragment of cyt *b*(216 - 394 bp) was successfully sequenced and analyzed for both species. Five haplotypes was found for the 41 *D. auricularis* specimens included. The most common haplotype, which characterized 38 of the specimens, was found across the distribution of species. Between six and seven mutational steps separated this common haplotype from the others.Haplotype diversity is low reflecting the large number of specimens sharing the common haplotype.The mantel test of *D. auricularis*showed a non-significant (but positive) linear correlation between genetic and geographic distances (r = 0.013;p = 0.41) indicating no isolation

by distance. Phylogenetic trees (Mr Bayes, NJ andMP)revealedtwo putative lineages within *D. auricularis* with sequence divergence values between these two clades ranged between 1.52% and 1.77%. Notwithstanding a lower sample size (N=26) and a shorter fragment analyzed (216 bp), more variation was detected in *G. paeba*with eleven haplotypes found. The two most divergent haplotypes are separated by 14 mutational step (6.48% uncorrected sequence divergence). Haplotype diversity was 0.4909 ± 0.1754 and nucleotide diversity 0.004714 ± 0.003846.A Mantel test showed non-significant but positive linear correlation ($r = 0.13, p = 0.16$) between geographic and genetic distance, supporting no isolation by distance. Phylogenetic analyses retrieved two clades with the sequence divergence between these clades ranging between 0.463 – 4.17%.Clade A is comprised of individuals from the Free State,Northern Cape and LimpopoProvinces. Clade B is comprised of individuals from Namibia, the Free State, Northern Cape and Eastern Cape Provinces. Overall, the two datasets are not congruent (for each species). For *D. auricularis*,morphometrics dataset recognize a single lineage and molecular dataset recognise two lineages. However, morphometric showed two OTUs and DNA data showed two lineages in *G. paeba*which does not have sympatric distribution. The habitat preferences were probably responsible for shaping the morphological and geneticalvariation observed in these two sympatrically distributed gerbil species.

Opsomming

Die doel van hierdie studie was om te beskryf en die phylogeographic strukture van *D. auricularis* en *G. paeba* vergelyk, en bepaal of die vier erkende subspesie is geldig deur gebruik te maak van tradisionele morphometrics en molekulêre data gebaseer op gedeeltelike rye van die mitochondriale sitochroom B (cyt *b*) geen. Die tradisionele morfometriese ontleding is gebaseer op 12 kraniale veranderlikes wat van 89 monsters van 54 plekke vir *D. auricularis* en 48 *G. paeba* monsters van 25 plekke geneem. Veranderlikes van beide mans en vrouens is gekombineer sedert univariate en meerveranderlike ontleding aan die lig gebring dat daar was geen seksuele dimorfisme in die twee spesies (Wilks se lambda, Λ = 0942, p = 0,78 vir *D. auricularis* en Λ = 0, 81, p = 0,82 vir *G. paeba*). Eenveranderlike analise het aan die lig gebring beduidende ouderdom variasie en slegs ouderdom klas II en III is wat gebruik word vir beide spesies (vir *D. auricularis*, Λ = 0713; p = 0,035 en *G. paeba*, Λ = 0, 748, p = 0.04). Meerveranderlike ontleding het aangedui dat daar nie 'n intra-bevolking variasie wat bevestig dat *D. auricularis* is' n monotipiese spesie. Maar, meerveranderlike resultate vir *G. paeba* stel voor dat drie hoof operasionele taksonomiese eenhede bestaan. Veranderlikes die onderskeid van hierdie operasionele taksonomiese eenhede is die breedte van die eerste molêre, skedel lengte en rostrale hoogte. Overall, morphometrics resultate dui daarop dat vier subspesies van *G. paeba* drie subspesies kan verminder word. Dit sluit *G. p. paeba* wat die wydste verspreiding en die bestaan van die monsters van die xeric en Mesic spesies [Namibië en Suid-Afrika (Noord-Kaap, Wes-Kaap en Oos-Kaap Provinsie)], *G. p. coombsi* bestaan uit monsters van die Soutpansberg gebied en *G. C. kalaharicus* bestaan uit individue uit Botswana, wat binne die Mesic gebied versprei is.

Die molekulêre aspek van hierdie projek is ook gebaseer op die museum materiaal van *D.auricularis* (N = 41 van 25 plekke) en *G. paeba* (N = 26 van 12 plekke). 'N fragment van cyt *b* (216 – 394 BP) was suksesvol volgorde en geanaliseer vir beide spesies. Vyf haplotypes was gevind vir die 41 *D. auricularis* monsters ingesluit. Die mees algemene haplotype, wat gekenmerk word 38 van die monsters, is gevind oor die verspreiding van spesies. Tussen ses en sewe mutasie stappe hierdie gemeenskaplike haplotype van die ander geskei. Haplotype diversiteit laag is dit die groot aantal monsters deel van die gemeenskaplike haplotype. Die

mantel toets *D. auricularis* het 'n nie-beduidende (positiewe) lineêre verband tussen genetiese en geografiese afstande (p = 0,41; r = 0,013) wat aandui dat geen isolasie deur die afstand. Filogenetiese stambome (Mnr Bayes, NJ en LP) het aan die lig gebring twee vermeende afstammelinge binne *D. auricularis* volgorde divergensie waardes tussen hierdie twee clades gewissel tussen 1,52% en 1,77%. Ten spyte van 'n laer steekproefgrootte (N = 26) en' n korter fragment ontleed (216 bp), is meer variasie bespeur in *G. paeba* met elf haplotypes word. Die twee mees uiteenlopende haplotypes word geskei deur 14 mutasie stap (6,48% nie-reggestelde volgorde divergensie). Haplotype diversiteit 0,4909 ± 0,1754 en nukleotied diversiteit 0,004714 ± 0,003846. 'N Mantel toets het getoon nie-betekenisvolle maar positiewe lineêre korrelasie (p = 0,16; r = 0,129) tussen die geografiese en genetiese afstand, geen isolasie deur die afstand te ondersteun. Filogenetiese ontleding opgespoor twee clades met die volgorde verskille tussen hierdie clades wissel tussen 0,463 – 4,17%. Clade 'n bestaan uit individue van die Vrystaat, Noord-Kaap en Limpopo provinsies. Clade B bestaan uit individue uit Namibië, die Vrystaat, Noord-Kaap en Oos-Kaap. Overall, die twee datastelle is nie kongruent (vir elke spesie). Vir *D. auricularis*, morphometrics dataset herken 'n enkele afkoms en molekulêre datastel erken twee geslagte. Maar, morfometriese gewys drie Otus en DNS-data het twee geslagte in die *G. paeba*. Die habitatvoorkeure was waarskynlik verantwoordelik vir die vorming van die morfologiese en genetische variasie in hierdie twee sympatrically versprei Gerbil spesies waargeneem.

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General introduction

1.1 Preamble

Rodents represent almost half of all known mammalian species and are distributed globally (Skinner and Chimimba 2005and references therein; Musser and Carleton, 2005). In southern Africa rodents occur in the two main habitats: mesichabitats characterised by temperate precipitationwith annual rainfall ranging between 230-725mm and arid habitats characterised by sparse vegetation and annual mean rainfall ranging between13 – 290 mm (Mucina and Rutherford, 2006; Skinner and Chimimba 2005). Within the family Muridae, this habitat preference dichotomy is evident at the subfamilial level. Of the seven murid subfamilies occurring in southern Africa, four (Acomyinae, Murinae, Cricetomyinae, Dendromurinae) have both arid and mesic distribution, two (Gerbillinae and Petromyscinae) have a largely arid range and one (Mystromyinae) have a mesic distribution (Skinner and Chimimba, 2005). Among these rodents, the widely distributed species have been shown to contain multiple lineages (e.g. Chimimba, 2001; Rambau e*t al*., 2003; Russo *et al*., 2010; Engelbrecht *et al*, 2011), probably as a result of local adaptations.

Since the distribution of small mammals depends on the climate and environment in which they occur (Skinner and Chimimba, 2005), it is important to investigate whether the divisions demarcated by their distribution within different biotic zones are similarly reflected in their genetic structures. Gerbils provide a perfect representation of arid dwelling rodents to test whether biotic (or biome) divisions that comprise arid regions may have the effect on the population structure of rodents' occupying these regions. In this study, two species from the subfamily Gerbillinae (Alston, 1876), *Gerbillurus paeba* (A. Smith, 1936) and *Desmodillus auricularis* (A. Smith, 1934), will be used to investigate whether subdivisions of the arid region are similarly reflected in their genetic (as inferred by mtDNA) and morphological (as inferred from cranial morphometrics) attributes.

1.2 Systematics of the Gerbillinae

The subfamily Gerbillinae (suborder Myomorpha and family Muridae) is one of the two subfamilies with a predominantly arid distribution. Globally, the subfamily has 14 genera and 110 species of which three genera and nine species occur in the arid region of southern Africa (Musser and Carleton, 2005; Skinner and Chimimba, 2005). Gerbils comprise a monophyletic group and their sister taxa in Africa are *Acomys* and *Lophuromys* (Chevret *et al.*, 1993; Jansa and Weksler, 2004, Chevret and Dobigny, 2005).

In southern Africa the subfamily is comprised of three genera: *Gerbilliscus* (African *Tatera*), *Gerbillurus* and the monotypic genus *Desmodillus* (Meester *et al.*, 1986; Musser and Carleton, 1993; Colangelo *et al.*, 2005 and 2007). Cytogenetically all four species of *Gerbillurus* are distinguished from *Gerbilliscus* by the structure of Y and X chromosomes and diploid numbers (Qumsiyeh *et al.*, 1991). Phenotypically, they are distinguished by the morphology of the incisors (Pavlinov, 2001). Cytogenetic and morphological data suggest that *Taterillus*, *Gerbillurus* and *Tatera* belong together in the tribe Taterillini (Chevret and Dobigny, 2005 and reference therein). The genera *Gerbilliscus* and *Tatera* (Asian) are not closely related and *Gerbillurus* is evolutionarily closer to *Gerbilliscus* based on skull morphology (Pavlinov, 2001), chromosomal data (Qumsiyeh, 1986; Qumsiyeh *et al.*, 1991), DNA/DNA hybridization and molecular sequence (Chevret and Dobigny, 2005). In summary, the genus *Tatera* is restricted to Asia while *Gerbillurus* and *Gerbilliscus* have an African distribution.

1.3 Rationale

Of the arid dwelling gerbils occurring in South Africa, three have a restricted distribution (localized such as *G. tytonis*, *G. vallinus* and *G. setzeri*) and two (*D. auricularis* and *G. paeba*) have wider distributions (Dempster *et al.*, 1998; De Graaff, 1981; Musser and Carleton, 2005; Wilson and Reeder, 2005; Skinner and Chimimba, 2005). The focus of this comparative study will be on these widely distributed species partly because of several attributes: firstly, they occur sympatrically throughout their geographic range, secondly, they have somewhat different life histories and thirdly, they have different reproduction systems (De Graaff, 1981). And, although

they are distributed sympatrically, it is interesting that no subspecies are recognized for *D. auricularis* while four are recognized in *G. paeba*.

Several arid distributed species occurring in southern Africa have been investigated using a variety of methods to uncover geographic structuring within their range. However, no phylogeographic study has to date been undertaken for the two gerbil species. This is in contrast to the population genetic analysis that has been recorded for several mammalian and non-mammalian taxa in the Southern African sub region (see for example *Pronolagus rupestris,* Matthee and Robinson, 1996; *Aethomys granti,* Chimimba *et al*. 1998;*Aethomys/Micaelamys namaquensis,* Chimimba, 2001 and Russo *et al*., 2010;*Agama atra,* Matthee and Fleming, 2002; *Elephantulus edwardii,* Smit *et al.,* 2007; *Rhabdomyspumilio,* Rambau *et al.,* 2003; *Macroscelides proboscideus,* Smit *et al.,* 2010; *Myotomys unisulcatus,*Edwards *et al*., 2011 and *Otomys irroratus,* Engelbrecht *et al*., 2011). Most of these species have overlapping ranges to the species used in the present study, and as such are useful for comparative purposes. In addition to investigating phylogeography, this study will also establish whether the geographic barriers identified in southern African (e.g. Western escarpment, the Cape Fold Mountains, The Orange River which forms the border between Namibia and South Africa) and biomes/biotic vegetation occurring throughout the range of these species will have a similar impact on the genetic or morphology of*G. paeba* and *D. auricularis*.

1.4 General biology of study animals

(a) Life history and taxonomy

Gerbils are distinguished from other subfamilies within the Muridae by their pelage color (tawny colored upper parts and white under parts), relatively large eyes (an adaptation for nocturnal existence), and enlarged bullae comprising at least 25% of greatest skull length (De Graaff, 1981). Their short forelimbs are adapted to burrowing and well-developed hind limbs are suited for saltatorial movement. Unlike other murines, all gerbils have two or three molar teeth comprising oval shaped ringsof enamel and an absence of rows of cusps observed in murines (De Graaff, 1981; Skinner and Smithers, 1990; Colangelo*et al*., 2007).

Gerbil species are easily distinguishable from each other based on body size, phenotype, and skull features. The Cape short-tailed gerbil, *D. auricularis* is an average sized gerbil (weighs about 53g and have total length of ~ 200mm) and can be distinguished from other gerbils by the presence of white spots behind the ears (see Table 1; De Graaff, 1981). The zygomatic plates in their skull are short and not as well developed as in the genus *Gerbilliscus* (De Graaff, 1981; Wilson and Reeder, 2005; Skinner and Chimimba, 2005). Further, differences in body color in *D. auricularis* has been recorded in different individuals from the same locality in Botswana; some were brownish-buff, cinnamon-buff, grey-brown, and others had grey-brown color on the back, and the front parts lighter in color with the exception of the dorsal part which is always pure white (Smithers, 1971). The monotypic *Desmodillus* has several synonyms throughout its range probably reflecting local vernacular as opposed to diagnostic characters (Table 1; De Graaff, 1981; Meester *et al*., 1986). In fact, several *Desmodillus* subspecies that were previously described predominantly based on color were not accepted or recognized as valid because in some areas all morphs were syntopic (Smithers, 1971 and reference therein).

Unlike the monotypic *Desmodillus*, the genus *Gerbillurus* contains four species and all have a tail that is longer than the body and head length plus total body length less than 120 mm. The soles of their hind feet are slightly or entirely haired and the central narrow part uncovered. The zygomatic plates are projected less forward compared to other gerbils. *Gerbillurus* consists of three subgenera: *Progerbillurus* (*G. paeba*), *Paratatera* (*G. tytonis*), and *Gerbillurus* (*G. vallinus* and *G. setzeri*) which differ in size and habitat preference. Of the four species composing *Gerbillurus*, *G. paeba* occurs widely in the arid regions of southern and the other three occur in Namibia (De Graaff, 1981; Dempster *et al.*, 1998; Wilson and Reeder, 2005; Skinner and Chimimba, 2005).

Among gerbils, *G. paeba* is smallest in body size (adult weighs less than 30g) and shortest in body length (mean total body length is 210 mm; Smithers, 1971) compared to other *Gerbillurus* species. Its distinguishing phenotypic feature is the tufted tail, which is longer than the body and head length. Further, the ear bullae are flattened with poorly developed posterior parts. The species is divided into four subspecies: *G. p. coombsi*,*G. p. paeba*, *G. p. exilis*, and *G. p. infernus*, which are differentiated according to their geographic locations (Table 1, Fig. 1A; Meester *et al.*, 1986), and subtle differences in their social structures (see below).

Table 1: List of *G. paeba* subspecies and their type localities. *D. auricularis* has no recognized subspecies however; several synonyms have been proposed (De Graff, 1981; Meester *et al*., 1986; Wilson and Reeder, 2005).

Species name	Subspecies names	Type locality
Gerbillurus paeba[1] (A. Smith, 1836)	*G. p. paeba*	North of Latakoo, Vryburg, Northern Cape
	G. p. exilis (Lundholm, 1955)	Alexandria district, Eastern Cape Province.
	G. p. coombsi (Roberts, 1929)	Waterpoort, Soutpansberg in Limpopo Province.
	G. p. infernus (Shortridge and Carter, 1938)	Skeleton coast, northern Namibia
Desmodillus auricularis[2](A. Smith, 1834)	None recognized	Kamiesberg, Namaqualand, Northern Cape

[1]Synonyms of *G. paeba*: *Gerbillus paeba* (A. Smith, 1836), Country beyond Latakoo, Vryburg; *Gerbillus tenuis* (A. Smith, 1842), North of Latakoo; *Gerbillus calidus* (Thomas, 1918), Molopo, west of Morokwen; *Gerbillus paeba broomi*(Thomas, 1918), Port Nolloth; *Gerbillus swalius* (Thomas and Hinton, 1925), Namibia, northwest of Windhoek; *Gerbillus swalius oralis*(Thomas and Hinton, 1925), Namibia, Kuiseb River from Walvis Bay, Namib Desert; *Gerbillus swalius leucanthus* (Thomas, 1927) Namibia, Ondongwa, Ovamboland; *Gerbillus calidus kalaharicus* (Roberts, 1932)Botswana, Gomodimo Pan; *Gerbillus paeba mulleri* (Roberts, 1946), Western Cape, Eendekuil; *Gerbillus paeba swakopensis* (Roberts, 1951), Namibia, Swakopmund.

[2]Synonyms of *D. auricularis*: *Gerbillus auricularis* (A. Smith, 1834), Little Namaqualand, Kamiesberg; *Gerbillus brevicaudatus* (Cuvier, 1838), Cape of Good Hope; *Meriones caffer* (Wagner, 1842), South Africa; *Desmodillus auricularis pudicus* (Dollman, 1910), Botswana, Kalahari, Lehututu; *Desmodillus auricularis robertsi* (Lundholm, 1955),Namibia, Sesfontein, Kaokoveld; *Desmodillus auricularis shortridgei* (Lundholm, 1955), Eastern Cape Province, Port Elizabeth; *Desmodillus auricularis hoeschi* (Lehmann, 1955), Namibia, Okatjongeama; *Desmodillus auricularis wolfi* (Lehmann, 1955), Namibia, Vogelweide.

(b) Social structure and reproduction

The two study species have different social structures. On the one hand, *D. auricularis* is communal and lives in colonies throughout their distribution (Nel, 1967), however, there is also evidence indicating that they areasocial in the Kalahari Desert (Nel, 1975), and males and females have antagonistic encounters (Dempster *etal*., 1993). Variation in social structure between geographic areas may be indicative of either local adaptation or evidence of multiple lineages. In contrast, all subspecies of *G. paeba*are solitary migratory species with males travelling longer distances than females during foraging (Louw, 1972; Smithers, 1971). *Gerbillurus paeba* are highly aggressive to each other and display intraspecific agonistic behavior (De Graaff, 1981; Perrin *et al.*, 1999; Perrin and Boyer, 2000). *Gerbillurus p. exilis* has almost identical ethological similarities to *G. p. paeba* except it displays mild aggression compared to the latter (Dempster and Perrin, 1989).

Although*D. auricularis* and *G. paeba* display slightly different ethological patterns, they also have different physiological adaptations.For instance, *D. auricularis*can survive prolonged periods without access to water and consequently produce highly concentrated urine (5.5 $mOsm.kg^{-1}$)(Perrin and Curtis, 1980). Further, they have efficient thermoregulation ability and can control normal body temperature at different conditions (Skinner and Chimimba, 2005 and reference therein), while*G. paeba* have an efficient renal function and regulate body temperature by producing additional heat through non-shivering thermogenesis (Perrin *et al*., 1999). Other differences between the species include breeding systems: *D. auricularis* reproduce throughout the year and have an average litter size of 3.9 (Smithers, 1971). On the other hand, *G. paeba* is a seasonal breeder,although Smithers (1971) found that they are opportunistic aseasonal breeders in Botswana. In the wild the average number of foetuses per female in *G. paeba* is three to four (Smithers, 1971) and in captivity the average litter size is 4.6 (Dempster and Perrin, 1989). Females of *G. paeba*wean their offspring after 28-30 days (Dempster and Perrin, 1989; Perrin *et al*., 1999).

There are also slight differences in their diets with *D. auricularis*being predominantly granivorous (seeds of *Tribulus terrestris* are common in their diet) although in the Namib Desert

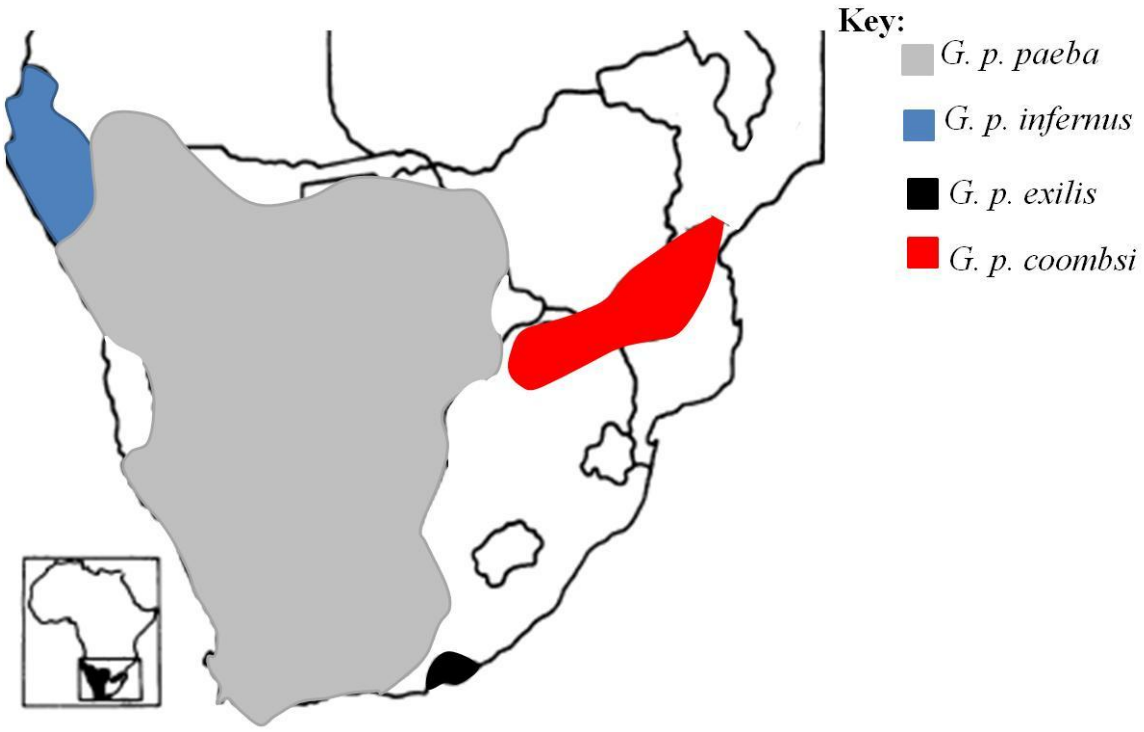
they are omnivorous (Nel, 1967). In contrast, *G. paeba* is predominantly omnivorous (Louw, 1972; Smithers, 1971).

(c) Distribution and habitat

In addition to reproduction and diet differences, both species have different habitat preferences. *Desmodillus auricularis* live in complex and wide burrow structures and can be found in the open ground (Nel, 1975). However, *G.paeba* live in simple burrows with only one entrance which is hidden under vegetation and not closed with sand (Downs and Perrin, 1989). They occur on sandy soil habitat with little grass cover. Subspecies of *G. paeba*have diverse environment preferences, for instance *G. p. coombsi*favors sandy soil in savannas while *G. p. exilis*favors dune valleys to dune crowns of the Namib Desert (Perrin *et al*., 1999; Skinner and Chimimba, 2005).

Generally, both species are distributed sympatrically in arid regions of southern Africa (Fig. 1A and B). In South Africa, both occur in the Central, Western and Northern Cape and the Free State Provinces. Their distribution extends to Namibia (except in the northeast) and southern parts of Botswana. The distribution of *G. paeba* also includes the northern region of the Limpopo Province (Fig. 1A, De Graaff, 1981; Skinner and Chimimba, 2005).

A



B

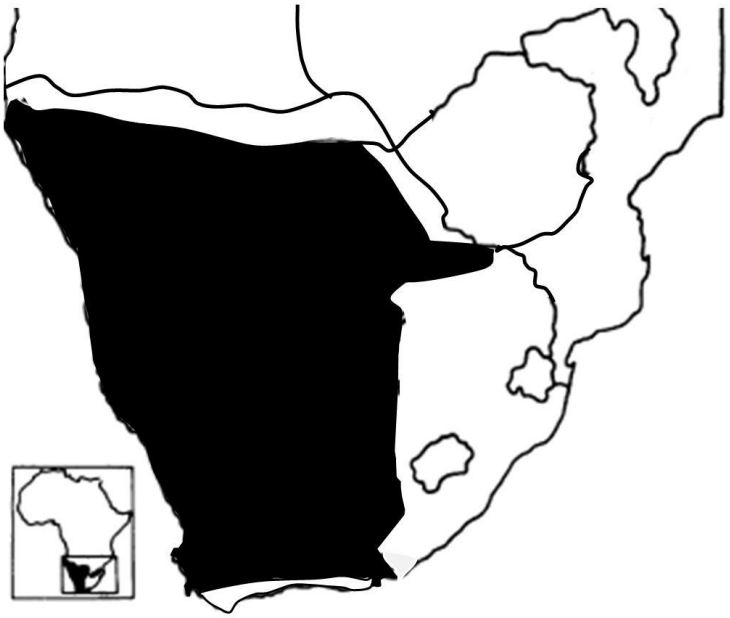


Figure 1: Distribution of *G. paeba* (A) and *D. auricularis* (B) in southern Africa. The four recognized subspecies of *G. paeba* are indicated in different shades (redrawn from Perrin *et al.*, 1999; Skinner and Chimimba, 2005).

1.5 Biotic Zones of Southern African arid region

The distribution of the two species spans five of the Southern African biotic zones: Southern Savanna Woodlands, Southern Savanna Grassland, South West Arid, Fynbos Zone and Namib Desert (Smithers, 1983). The mean annual rainfall in these biotic zones ranges from <125 mm (Namib Desert) to a maximum of 3000 mm in winter (e.g.Dwarsberg). As a whole, the Southern Savanna Woodland is wetter in the eastern regions (900 mm), than the Fynbos Zone in general (200 mm) (Smithers, 1983). The vegetation in these areas is mainly succulents including dwarf shrub, woodlands and grassland (Smithers, 1983; Mucina and Rutherford, 2006). These succulents supplement the water intake for the rodents occurring in these areas (De Graff, 1981). Specific vegetation types characterize the biotic zones of this subregion with clear boundaries separating them (Smithers, 1983; Mucina and Rutherford, 2006).

1.6 Morphometrics

Morphometric data are useful in inferring evolutionary morphological patterns between and within taxa (Rohlf and Marcus, 1993; Roth and Mercer, 2000) and have been used extensively to resolve the taxonomy and systematics of small mammals, including rodents. Generally, morphometrics analysis quantifies shape variation within and among organisms (Rohlf and Marcus, 1993). It involves the analysis of shape change and evolution usually to address developmental and evolutionary questions relating to shape change during growth (Rohlf and Marcus, 1993; Parsons *et al*., 2003).Two main approaches are used: traditional morphometrics analysis and geometrics morphometric analysis. The former involves linear measurements which provide mostly size of the characters while geometric morphometricsallows important insight into the evolution of morphological development and systematics, for instance dietary specialization (Van Cakenberghe *et al*., 2002). Geometric morphometric analysis includes both size, shape components of diversity, and involves relationship between taxa. Point coordinates or landmarks taken using specimens images recorded in two or three dimensions are used in geometric morphometric analysis as data.

Traditional morphometrics uses univariate and multivariate approaches to analyze sets of quantitative morphological variables (Marcus, 1990; Reyment, 1991). These variables are in fact

measurements of distances between two identifiable points or landmarks on the surface of specimen crania and are often measured with calipers. The patterns of variation within and among samples are statistically analyzed using principal components analysis (PCA), factor analysis, canonical variants analysis (CVA), and discriminant function analysis (DFA). However, there are several shortcomings associated with traditional morphometrics. Mainly, the aspect of shape variation is lost in the use of linear distance measurements (Adams *et al.,* 2004). Despite these shortcomings, traditional morphometrics remain useful in species delimitation (see e.g. Bates, 1985; Robinson and Dippenaar, 1987; Chimimba and Dippenaar, 1995; Chimimba *et al*., 1999; Faleh *et al*., 2010) and identification of cryptic species (see e.g. Dippenaar *et al*., 1993; Jackson and Van Aarde, 2003; Bronner *et al*., 2007).

1.7 Phylogeographic population structure

Phylogeography as a field has been set apart from classical population genetics by dealing explicitly with a species' history and the spatial distributions of gene lineages (Knowles and Maddison, 2002). Phylogeography allows assessment of historical scenarios which caused the present-day spatial arrangements of organisms. For instance, it allows insight into factors shaping an evolving population such as demographic changes (population expansions and reduction), fragmentation or dispersal. These attributes have a direct impact on the genetic structure of a species (Hare, 2001; Knowles and Maddison, 2002).In this study comparative phylogeography study will be undertaken, whichis the study that tests whether taxa that have a sympatric distribution share common evolutionary, demographic, and distributional histories that could produce shared intraspecific phylogeographic patterns.Comparative phytogeography evaluates whether the phylogeographic structures of the sympatric sister species or co-distributed taxa result from recently derived differences or from prehistorical processes, ecological preferences and dispersal abilities (Gutiérrez-García and Vázquez-domínguez, 2011 and reference therein).

Phylogeography can be investigated using different molecular markers such as allozymes as well as sequence data from a variety of loci including the Y chromosome, mitochondrial and nuclear markers. The relatively faster mutation rate allows these markers to unravel inter- and intraspecific variation even when morphological markers cannot distinguish taxa because of slow rates of change (Avise, 2000; Mora *et al.,* 2006). The marker of choice for investigating phylogeography is mtDNA, which has been used extensively because of several attributes.

MtDNA is maternally inherited, evolutionarily conserved, variable and can be amplified using universal primers (Brown *et al*., 1979; Irwin *et al.,* 1991; Lansman *et al*., 1983; Avise *et al*., 1989; Avise, 1994). Implicitly, methods used in phylogeographic analyses examine the phylogenetic relationships among geographically distinct populations (population is used here as a synonym for sampling locality), and make inferences about evolutionary diversification of these populations (Polly, 2003).

A combination of morphological and molecular data is useful in identifying groupings, and to make accurate phylogenetic predictions of spatial structuring of a species (Nice and Shapiro, 1999). The sole use of cranial morphometrics will only reveal the role of morphological changes in the species and this might not reveal the true extent of variation within species (Edwards *et al*., 2011 and references there in). In order to address this mtDNA cyt *b* was included in the analysis because it is variable (Simmons and Hand, 1998 and reference there-in) and therefore may provide estimates of genetic diversity in species. A combined approach has been used successfully in defining cryptic species, particularly when dealing with widely distributed species where multiple lineages may occur such as *Praomys* (Nicolas *et al*., 2005), *Jaculus jaculus* (Faleh *et al*., 2010) and*Otomys irroratus* (Engelbrecht *etal*., 2011).

1.8 Utility of museum voucher specimens

This study is largely based on museum specimens because biological collections are valuable sources of information (for discussions see Campbell *et al.,* 2011). These collections provide almost complete sampling of the diversity partly because they represent extended periods of collections. Further, the use of museum collection in this study eliminated the costs associated with fieldwork.

Museum collections are particularly useful for investigations involving linear measurements (Chimimba, 2001); however, their utility in genetic studies may be problematic mainly because of DNA degradation which is dependent on treatment and storage of specimens. This is partly due to co-purification of substances that may inhibit enzyme activity leading to incomplete digestion of tissue and subsequent inadequate amplification of DNA. However, improvements in the DNA extraction techniques have now made it possible to extract DNA from ancient

specimen's older than12, 000 years. This is demonstrated by an increase in the number of studies based on material accessed from museums (Krings*et al*., 1997; Serre *et al*., 2004), especially when the gene fragments are small (200-300 bp; Casas-Marce *et al*., 2010).

1.9 Aims and objectives of this study:

Against this background, this study compares patterns of geographic variation between*D.auricularis* and *G. paeba*. Cranial measurements were used for morphological variation and mtDNA was employed for genetic variation across distribution of these two species.

Null Hypotheses

1. Given that the distribution of the two target species span several of the biotic zones of the southern Africa, morphology of the species may vary with biotic zones possibly due to local adaptation (and / or genetic) or geographic (vegetational) barriers for the species.
2. Given that the target species are largely sympatrically distributed they will exhibit the same genetic structure(notwithstanding the structure may be controlled by phylogeny more than ecology).

These hypotheses will be tested in two sections: first, morphometrics variation will be assessed (Chapter 2) followed by genetic analysis (Chapter 3). Both analyses will be evaluated across the range of the respective species.

Chapter 2

Intraspecific morphometric variation of the Cape short-tailed gerbil, *Desmodillus auricularis* and the hairy-footed gerbil, *Gerbillurus paeba*

2.1 Introduction

Gerbillurus paeba and *D. auricularis*occur sympatrically throughout most parts of their xeric distribution and they are easily distinguished in the field. Of the two species, *D. auricularis* is larger with a short tail and white spot behind the ears, while *G. paeba* is the smaller and is distinguished by a tufted tail that is longer than the total body length (De Graaff, 1981; Skinner and Smithers, 1990, Skinner and Chimimba, 2005). Other diagnostic phenotype differences include bullae size (larger in *D. auricularis*), zygomatic plates (well developed in G. paeba), maxillary molars and mandibular incisors (both distinctly ridged in *G. paeba*), and the rostrum (long in *G. paeba*) (De Graaff, 1981; Skinner and Chimimba, 2005).

To date, only one study has investigated the level of intraspecific cranial variation in*D. auricularis* and *G. paeba*. However, this was based on individuals from one locality, Gorasis in the Namib Desert (Matson and Christian, 1996). In that study they tested the "niche variation" hypothesis (van Valen, 1965) in coexisting populations to identify whether the species occupying a broader niche (*D. auricularis*) was morphologically more variable than the species occupying a smaller niche (*G. paeba*). Data from eight cranial variables revealed more size variation in *D. auricularis* than in *G. paeba*; however, this was in contrast with cranial shape measurements which were more variable in *G. paeba*. Matson and Christian (1996) concluded that the different degree of correlation among cranial measurements of *G. paeba* and *D. auricularis* was correlated with differences in population growth rates and demographic seasonality. Although the current study does not represent an extension of work by Matson and Christian (1996), the latter suggests that there may be morphological variation across the range of the species. Further, differences in number of subspecies between *D. auricularis* and *G. paeba* (Table 2; De Graaff 1981; Meester *et al*., 1986) provide sufficient grounds to investigate variation across the range of respective species, which may be evident through analysis of cranial variables using statistical tools (Marcus, 1990; Rohlf and Marcus, 1993; Marcus and Corti, 1996).

Morphometric variables that are particularly useful in mammals include those associated with mastication (molar tooth raw; Smith, 1993) or orofacial functional set (Chimimba and Dippenaar, 1995) and hearing for predator detection (bullae size; Lay, 1972) or neurocranial functional set (Cheverud, 1982; Chimimba and Dippenaar, 1995) and a combination of neurofacial/orofacial functional sets (skull length; Chimimba and Dippenaar, 1995). This approach has been used widely to investigate infraspecific/intraspecific variation in several gerbil species (Bates, 1985; Granjon, 2005; Matson and Christian, 1996; Faleh *et al*., 2010; Khajeh and Meshkani, 2010).

In southern Africa, data derived from cranial morphometrics of small mammals has been useful in taxonomic revision of genus *Aethomys*, (Chimimba and Dippenaar, 1995; Chimimba *et al*., 1999; Chimimba, 2001) and southern African Leporidae (Robinson and Dippenaar, 1987), and identification of cryptic species (*Mastomys natalensis* and *M. coucha*, Dippenaar *et al*., 1993; Jackson and Aarde, 2003; Bronner *et al*., 2007). Of these taxa, *Aethomys namaquensis* has a range which closely mirrors that of the two study species. Cranial morphometric analysis revealed that *A. namaquensis* has four morphometrically distinct groups which were interpreted as subspecies grouped according to phytogeographical regions of southern Africa and which differ in both cranial shape and size (Chimimba, 2001). While the range of *Elephantulus rupestris* is identical to that of the two study species, it prefers rocky habitats which are not connected and homogenous (Smit *et al*., 2010 and reference therein), while *D. auricularis* and *G. paeba* prefer sandy soil substrate habitat (Christian, 1980; De Graaff, 1981).

Against this background, the aim of this morphometric analysis is twofold:

1. To investigate patterns of morphological variation of *D. auricularis* and *G. paeba*across the biotic zones of the subregion.

2. To determine whether the currently recognized four subspecies o*f G. paeba* havediagnostic morphological differences.

2.2 Materials and methods

2.2.1 Specimens

In total two hundred and fifty five specimens (N=116 of *D. auricularis* and N= 139 of *G. paeba*) were obtained from the small mammal collection of the Ditsong National Museum of Natural History (formerly Transvaal Museum). The specimens originated from a combined 63 localities distributed in South Africa, Namibia, and Botswana (Fig. 2.1A and B; Appendix 1).

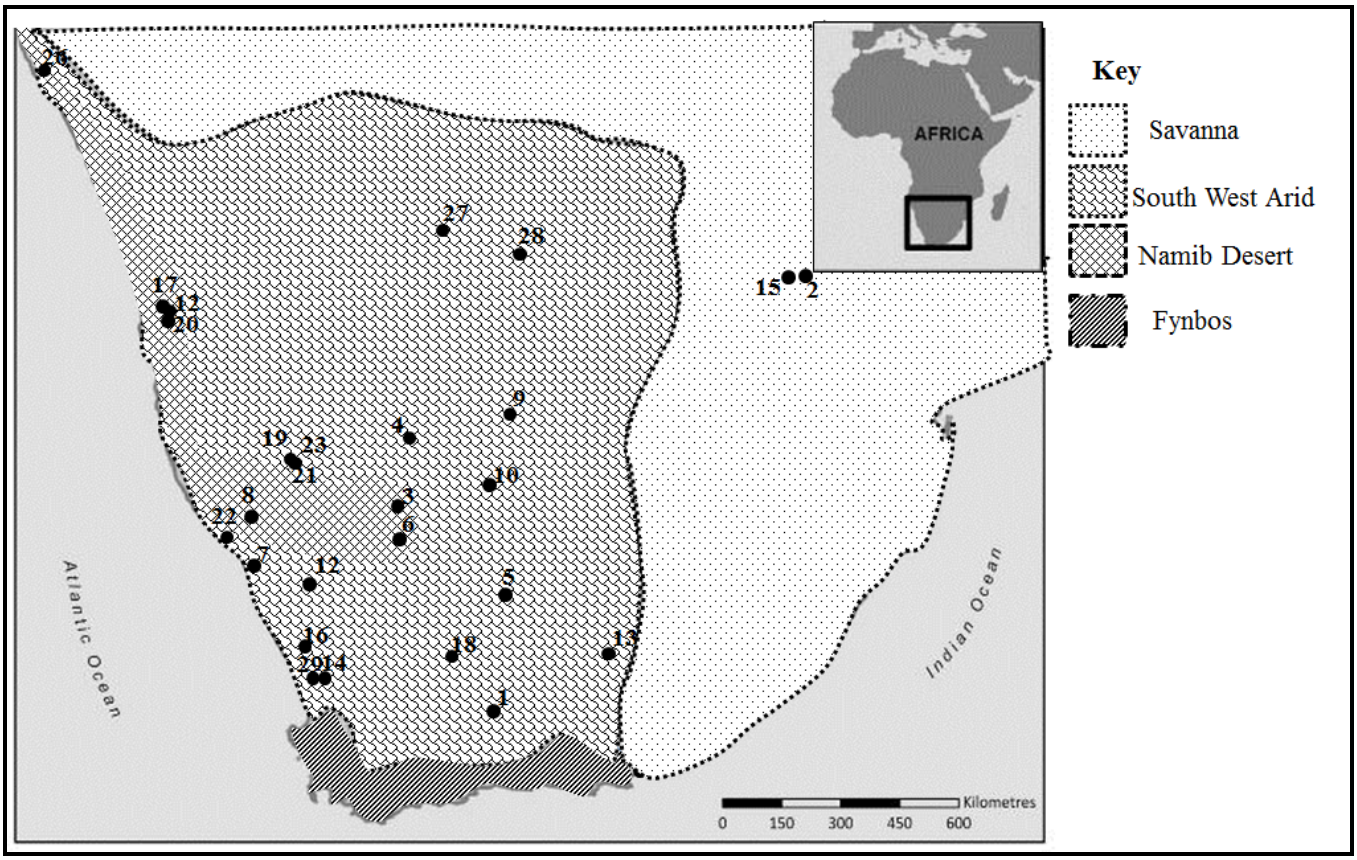


Figure 2.1A: Southern Africa map with 29 localities of *G. paeba* specimens used in this study. Numbers associated with the localities link to further information about the localities and the specimens in Appendix 1A. The boundaries of the biotic zones are indicated by differentshadings.

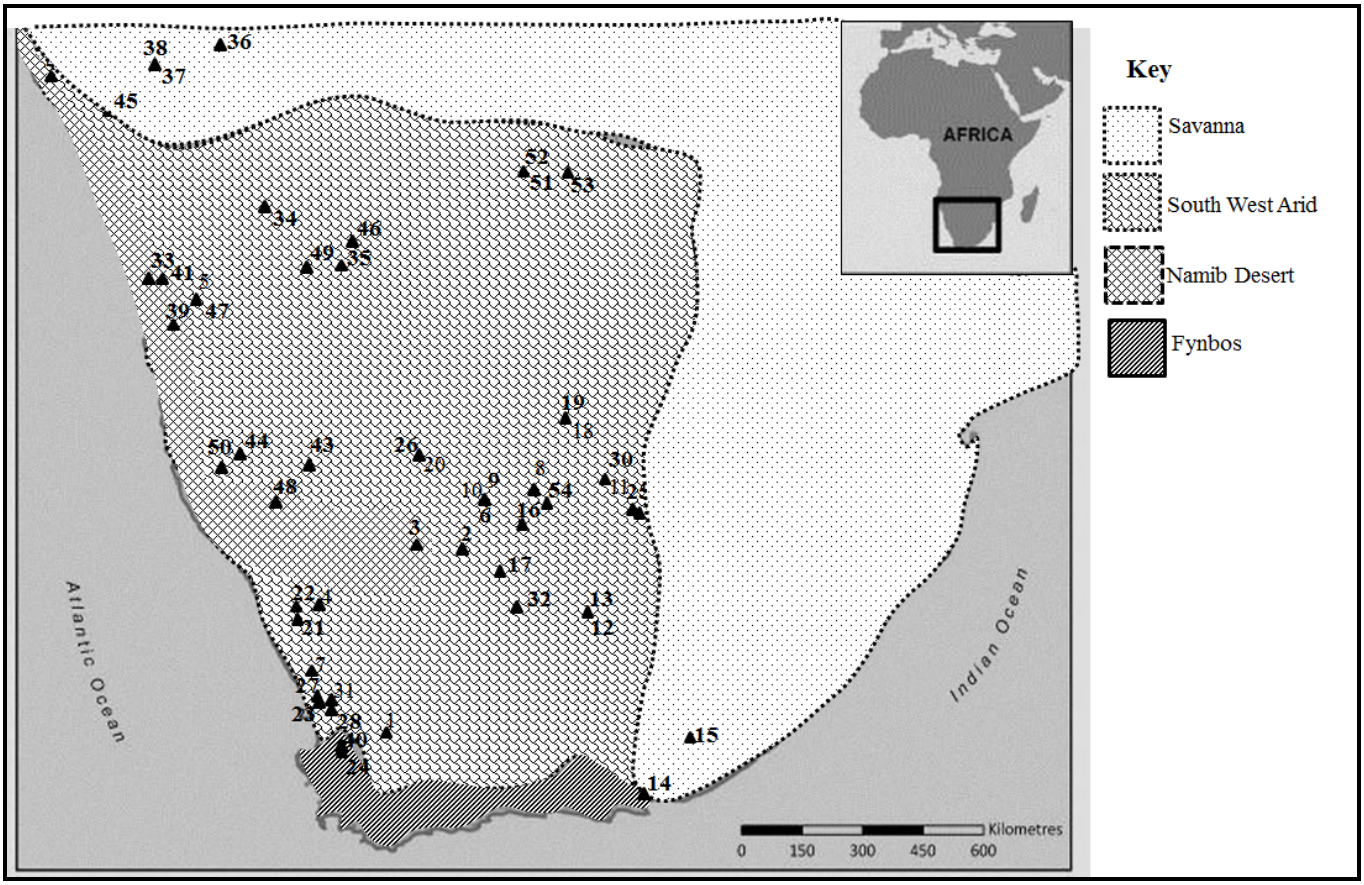


Figure 2.1B: Southern Africa map with 54 localities of *D. auricularis* specimens used in this study. Numbers associated with the localities link to further information about the localities and the specimens in Appendix 1B. The boundaries of the biotic zones are indicated by different shadings.

2.2.2 Cranial variable selection

Fourteen cranial measurements were taken using digital callipers to the nearest 0.01mm following Bates (1985) (Fig. 2.2; Table 2.1). Measurements were taken from the dorsal and ventral side of the cranium and mandible. The measurements used represent the following functional sets: (1) mastication or orofacial functional set, (2) hearing for predator detection or neurocranial functional and (3) the mixture of neurofacial/orofacial functional set (Appendix 4). These measurements have also been used in other studies (e.g. Bates, 1985; Matson and Christian, 1996; Granjon, 2005; Khajeh and Meshkani, 2010).

The data were screened to detect outliers using univariate statistics (Zar, 1999; Tabachnick and Fidell, 1989, Appendix 2). Data were tested for normality using skewness (g1), kurtosis (g2) and Kolmogorov-Smirnov goodness of fit D-test (K-S) (Zar, 1999).

Table 2.1: Cranial variables measured from specimens of *D. auricularis* and *G. paeba*

Variables	Variable description
Greatest length of skull (GLS)	Greatest anterior-posterior diameter, from the tip of the nasal to the supraoccipital
Breadth of the brain case (BB)	This measurement was taken along the occipital
Interorbital breadth (IB)	Narrowest width across the interorbital region
Rostral width (RW)	Taken transversely immediately in the front of the zygomatic plates
Rostral height (RH)	Perpendicular from the point directly behind incisors
Rostral length (RL)	This is the length from the tip of the nasal to the antero-superior margin of the infraorbital foramen
Tympanic bulla length (TBL)	Greatest antero-posterior diameter, from the apex external to the hamular process to the point external to the paraoccipital process
Mandible length (ML)	From posterior surface of condylar process to anteroventral edge of incisor alveolus (excluding teeth)
Upper tooth row length (UTR)	From the front of the alveolar margin of the first molar to the back of the crown of the last molar
Breadth of maxillary M^1 (BM^1)	The measurement was taken across the first molar of the maxillary teeth
Palatal width (PW)	Across inner borders of the first molars of the maxillary teeth
Occipital height (OH)	From the midpoint of the occipital below the foreman magnum to the top of the lambda
Length of the mandibular cheek teeth (LMC)	From the front of the crown of the first molar to the posterior alveolar margin of the M^3
Height of the mandible (HM)	From dorsal edge of mandibular condyle to ventral edge of angular process

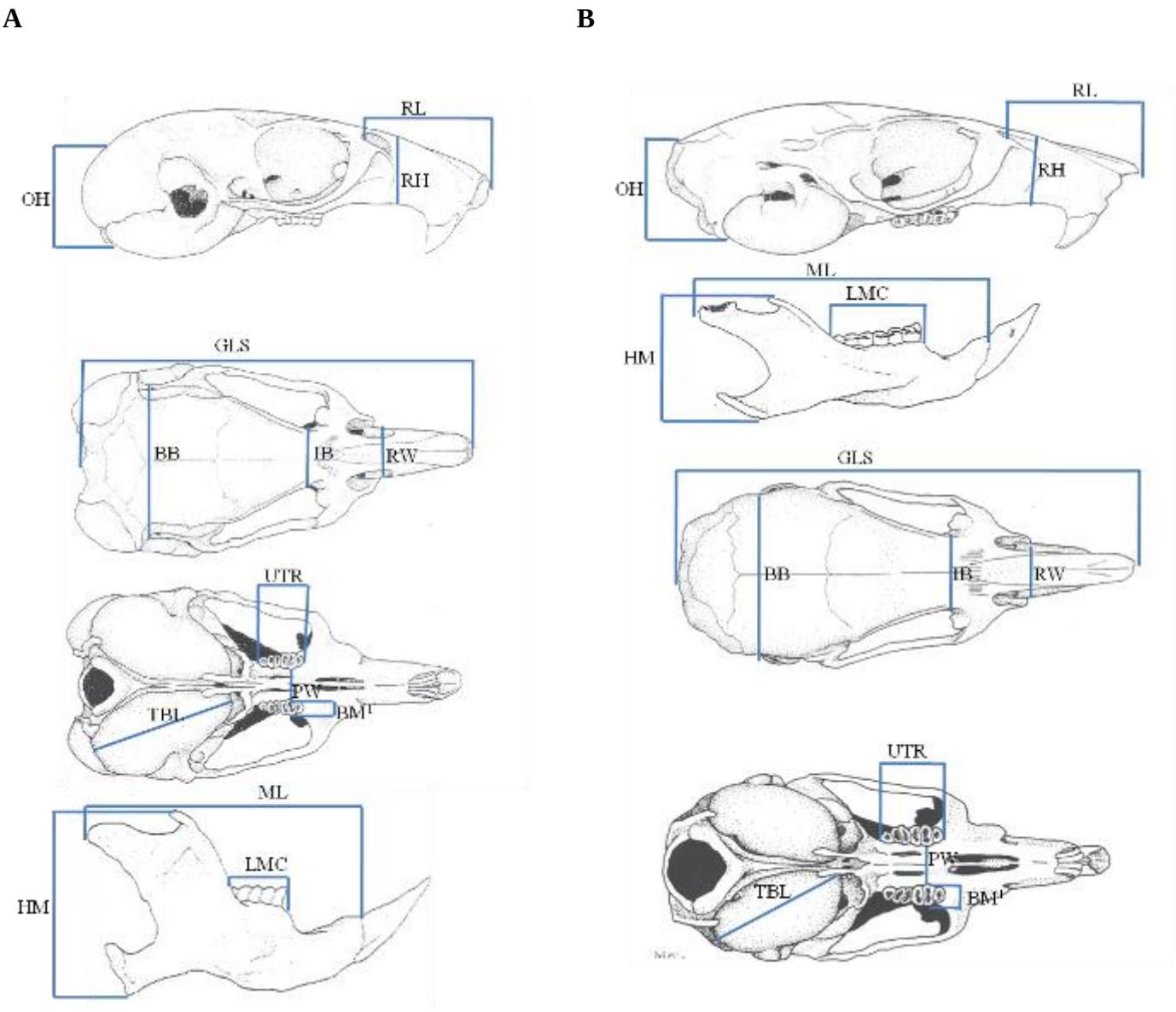


Figure 2.2: Schematic drawing of the dorsal and ventral side of the cranium and the mandible showing 14 cranial measurements used in phenetic analysis of both species (taken from Griffin, 1990): (A) = *D. auricularis* and (B) = *G. paeba*.See Table 2.1 for a full description of measurements.

2.2.3Measurement error

Measurement error (ME) is used in assessing variability introduced by the investigator and is a good indicator of whether variables were measured homologously consistently (Pankakoski *et al*. 1987; Taylor *et al*., 1990; Yezerinac *et al*. 1992). Measurement error was tested on 11 randomly chosen specimens for each species, from which 14 measurements were recorded on three separate

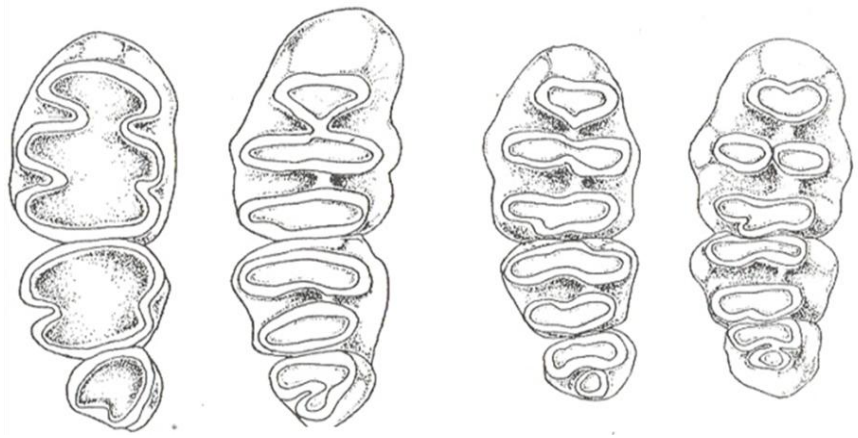
occasions, at two days interval. Percent measurement error was calculated using the sum of squared deviations from model II ANOVA method using the among- and within-individual variance components (Yezerinac *et al*., 1992). Mean within-individual coefficient variations were calculated for each individual and character using arithmetic means and standard deviations. The overall within-individual error (CV_{WI}) was calculated using the effects of different character means between eleven individuals for each character. The overall between individual errors (CV_{BI}) were calculated from the total variability of each of the three replicates of each character measurements of the eleven individuals (Pankakoski *et al.*, 1987).

Having removed problematic variables[3] during screening, PCA followed by cluster analysis based on Euclidean distance matrix (UPGMA) of *D. auricularis* were done using eight variables (GLS, IB, RW, RH, RL, TBL, ML and BM^1) and analysis of *G. paeba* were based on 12 variables (GLS, IB, RW, RH, RL, TBL, ML, BM^1 UTL, OH, LMC and BB).

2.2.4 Age class determinations

Molar tooth eruption and tooth wear are generally considered reliable estimators of relative age in small mammals (Bates, 1985; Chimimba and Dippenaar, 1994; Granjon, 2005, Hart *et al*., 2007; Bronner *et al*., 2007). The first maxillary molar teeth are the first to erupt and were used to age gerbils as previously done (e.g. *Gerbilliscus*, Bates, 1985; Colangelo *et al*., 2010; Granjon, 2005) Specimens were placed into four tooth wear classes based on the development of the second lamina of the first molar (M^1; Table 2.2; Fig. 2.3). In adult specimens, the laminae of the maxillary and mandibular molars are connected while the laminae of the younger ones are separated (Bates, 1985). After screening, all specimens and variables without statistical problems (which were normally distributed and have measurement error of less than 10%) were tested for age variation using PCA and ANOVA.

[3]Measurement error was greater than 10% in *G. paeba* for the following variables: HM (10.89%) and PW (13.7645%), and in *D. auricularis* for the following variables; HM (24.4657%), PW (11.6884%), OH (20.4363%), LMC (15.7788%), UTR (11.1378%) and BB (36.27655 %) (Appendix 3). These characters were discarded from further analyses.



Age class IV Age class III Age class II Age class I

Figure 2.3: Illustration of four tooth wear classes (I-IV) used to age specimens of both species (taken from Bates, 1985). Numbers represent age groups as described in Table 2.2.

Table 2.2: Tooth wear classes and their descriptions (Bates, 1985)

Tooth wear classes	Descriptions
Tooth wear age class I (youngest)	Lamina was divided by construction of enamel into two separate island and these were juveniles
Tooth wear age class II	Molars have only one island with a marked mesial narrowing and these were adults individuals
Tooth wear age class III	The narrowing of the molar is no longer apparent and the lamina becomes a single transverse plate. These were adult individuals
Tooth wear age class IV (oldest)	The second lamina was connected with both the first (M^1) and the third molar (M^3). These were the oldest individuals

2.2.5 Sexual dimorphism

Sexual dimorphism was tested using ANOVA using all 14 variable measurements to check whether different sexes should be analyzed separately in the analysis of geographic variation. To test sexual dimorphism without the potential influence of variation due to geographic variation, specimens from locations in close vicinity were assessed. Eleven individuals of *G. paeba* from several nearby localities, and 6 individuals of *D. auricularis* from Twee Rivieren in the Gordonia district were tested.

2.2.6 Assessment of geographic and species variation

Once the data had been screened (normality, measurement error, sexual dimorphism, age classes were determined) geographic variation was assessed. Principal component analysis (PCA) and canonical variates analysis (CVA) were used to assess geographic variation in relation to biotic zones, and subspecies variation. In the canonical variates analysis specimens were grouped according to different biotic zones of Southern Africa and for *G. paeba* it was also grouped to subspecies (following Smithers, 1983; Appendix 1).

2.3 Results

There was significant age variation (for *G. paeba*, Wilks' lambda, $\Lambda = 0, 748$; $p = 0.04$ and for *D. auricularis*, $\Lambda = 0{,}713$; $p = 0.035$). Juveniles (class I) formed a group to the exclusion of older individuals (class IV) using PCA and therefore both classes were discarded since this would introduce bias (Appendix 5 and 6). Consequently, only specimens with age class II and III were used for further analysis. However, there was no significant sexual dimorphism (Wilks' lambda, $\Lambda = 0, 81$; $p = 0.82$ for *G. paeba* and $\Lambda = 0{,}942$; $p = 0.78$ for *D. auricularis*) in either species (Appendix 5 and 6). Further analysis using UPGMA (Appendix 7) also revealed similar results, confirming previous observations(see Skinner and Smithers, 1990). Therefore, males and females were pooled for subsequent analysis.

2.3.1 Multivariate analysis of*D. auricularis*

The scatter plot of the first two principal components (PCs) did not reveal pronounced intra-population variation between different biotic zones. The variation obtained between the specimens of *D. auricularis* was 57.17 % for PC 1 and 11.969% for PC 2 (Fig. 2.4). The component loadings for the first principal component were correlated positively with PC 1; and there was a mixture of positive and negative loadings for PC 2 (Table. 2.3). Variables that contributed significantly on the x-axis (first component) were greatest skull length and mandibular length which contributed more than 40% variation. The variation in the second PC is largely due to breadth of the first molar which contributes 90% on the positive axis, while tympanic bulla length contributes 22% on the negative axis. The second component is probably correlated with shape (i.e. breadth of the first molar and bulla length) which are related to eating

and hearing. This suggests that the cranial size configuration between the samples of *D. auricularis* is correlated with both the mandibular and maxillary of the skull and PC 1 correlates with size. Overall, the major portions of differentiation in variables are associated with mastication (i.e. mandibular length and breadth of the first molar) and size of the skull.

While there is overlap of OTUs, individuals from the Savanna and Namib Desert clustered inside the SW Arid biotic zone. On the y-axis, they are distributed between ±2.4 and this shows that the breadth of the first molar is neither narrow nor wider. The breadth of the first molar has highest percent 89% correlation with second component. Individuals from the Namib Desert clustered from less than +0.6 towards the negative quadrant of the scatter plot, though they are scattered inside the South West Arid Zone individuals.

Canonical variates analysis scatter plot failed to group specimens according to different biotic zones of southern Africa (Fig. 2.5). The Namib Desert and Savanna are scattered inside the South West Arid Zone individuals. The character loading of both CVA's axes were correlated with both positive and negative loading (Table 2.4). Variables that contributed significantly on the x-axis were greatest skull length which contributed positively by 86% and mandibular length with contributed negatively by 42%.

Although there is no distinct geographic variation in cranial and mandibular morphology between individuals from different biotic zones (Wilks' Lambda: 0.4531; $P < 0.0038$), both principal component analysis and canonical variates analysis showed that most Namib Desert individuals are relatively smaller in size andalso show less variation in size.

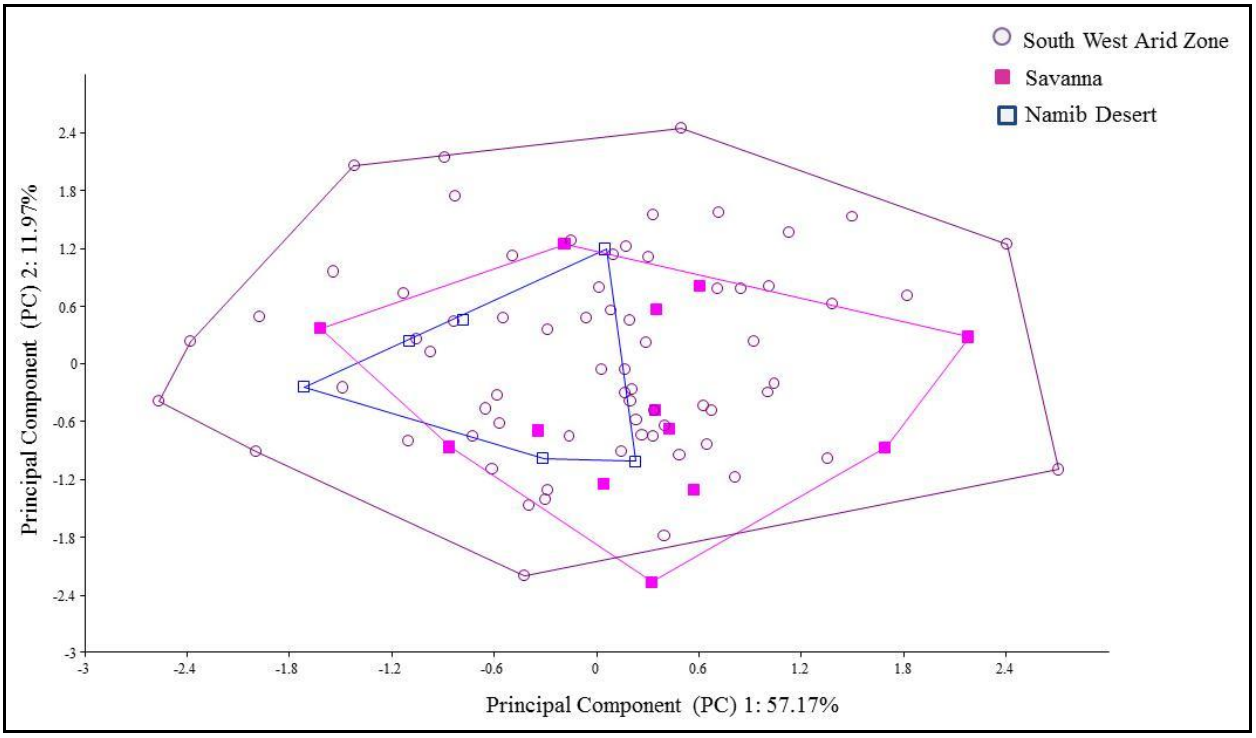


Figure 2.4: PCA **s**catter plot of the first two components (PC 1 and PC 2) based on eight cranial variables of 89 specimens of *D. auricularis*. Symbols (colored) identify individuals from different biotic zones.

Table 2.3: Variable component loadings of the first two principal components from PCA of *D. auricularis* with eigenvalues and total percent variation explained.

Variables	PC1	PC2
GLS	0.436845	-0.130373
IB	0.355065	-0.031415
RW	0.285173	0.239732
RH	0.378177	0.116468
RL	0.366734	-0.115747
TBL	0.368376	-0.222955
BM[1]	0.155744	0.908341
ML	0.405342	-0.150964
Eigenvalue	4.560809	0.957523
%variation explained	57.17	11.96904

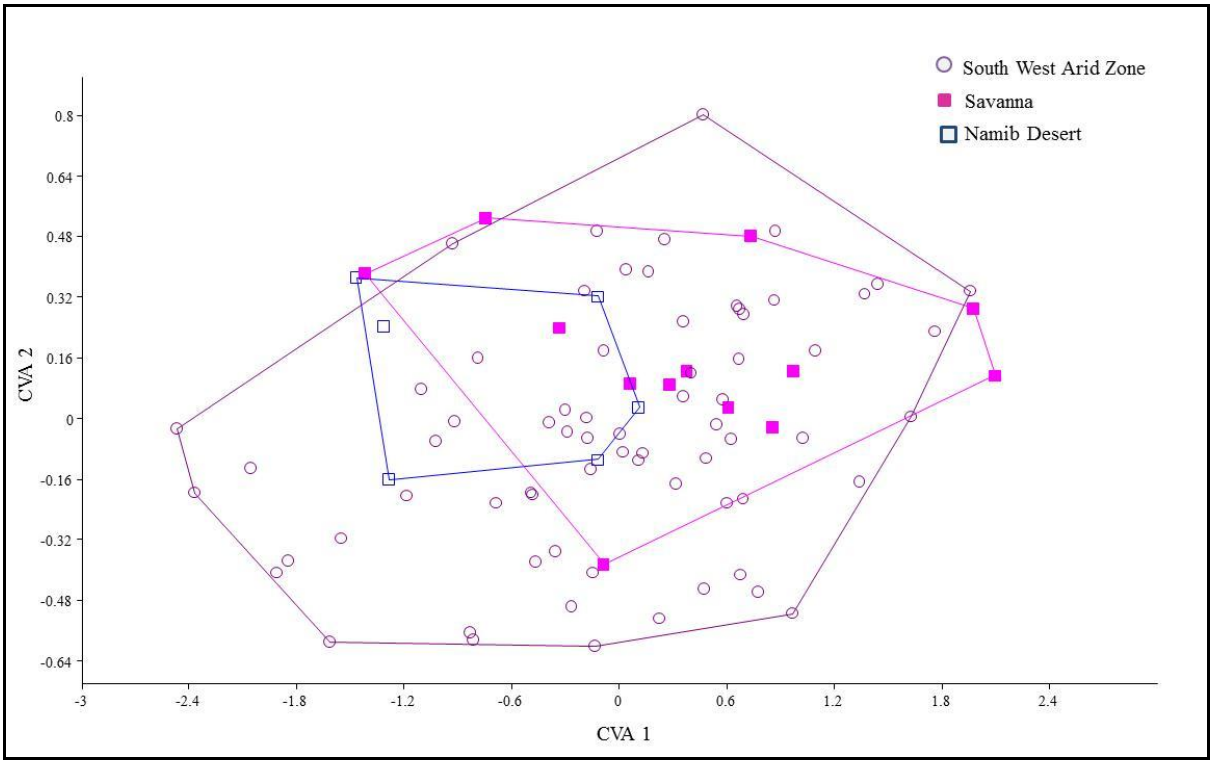


Figure 2.5: Canonical variates analysis scatter plot of the first two functional sets of *D. auricularis.* Symbols represent Operational Taxonomic Units (OTUs).

Table 2.4: Character loadings of the first two canonical variates analysis of *D. auricularis*

Variables	CVA 1	CVA 2
GLS	0.23746	-0.767844
IB	-0.07011	0.199762
RW	0.45897	-0.405001
RH	0.79610	-0.648420
RL	0.28082	0.635813
TBL	0.58869	0.965007
BM[1]	-0.60881	-0.291341
ML	-1.31826	0.204345

2.3.2 Multivariate analysis of *G. paeba*

The scatter plots for the first two principal components (Fig. 2.6) did not retrieve distinct intra-population variation between different biotic zones and subspecies (Fig. 2.7). Total percent variation in *G. paeba* for component 1 was 39.774 %, which indicates that there is relatively minor variation associated to size within and among individuals (Fig. 2.6 and 2.7). Percent variation explained by component 2 was 13.362% (Fig. 2.6 and 2.7) demonstrating that there was relatively minor variation related to shape within and between the specimens. The component loadings on first principal component were positive and a mixture of positive and negative on PC2 (Table 2.5 and 2.6). Variables that contributed significantly (> 30%) on the x-axis were greatest skull length and mandibular length. In contrast, the variation in the second component is due to breadth of the first molar (47.06%) and occipital height (51.78%).

Individuals from the South West Arid Zone overlap completely with other zones (Savanna, which include southern savanna grassland and woodland; and Namib Desert). However, the individuals from Namib Desert are mostly grouped from -1 to the positive side of the x-axis and other individuals are grouped almost all over the scatter plot.

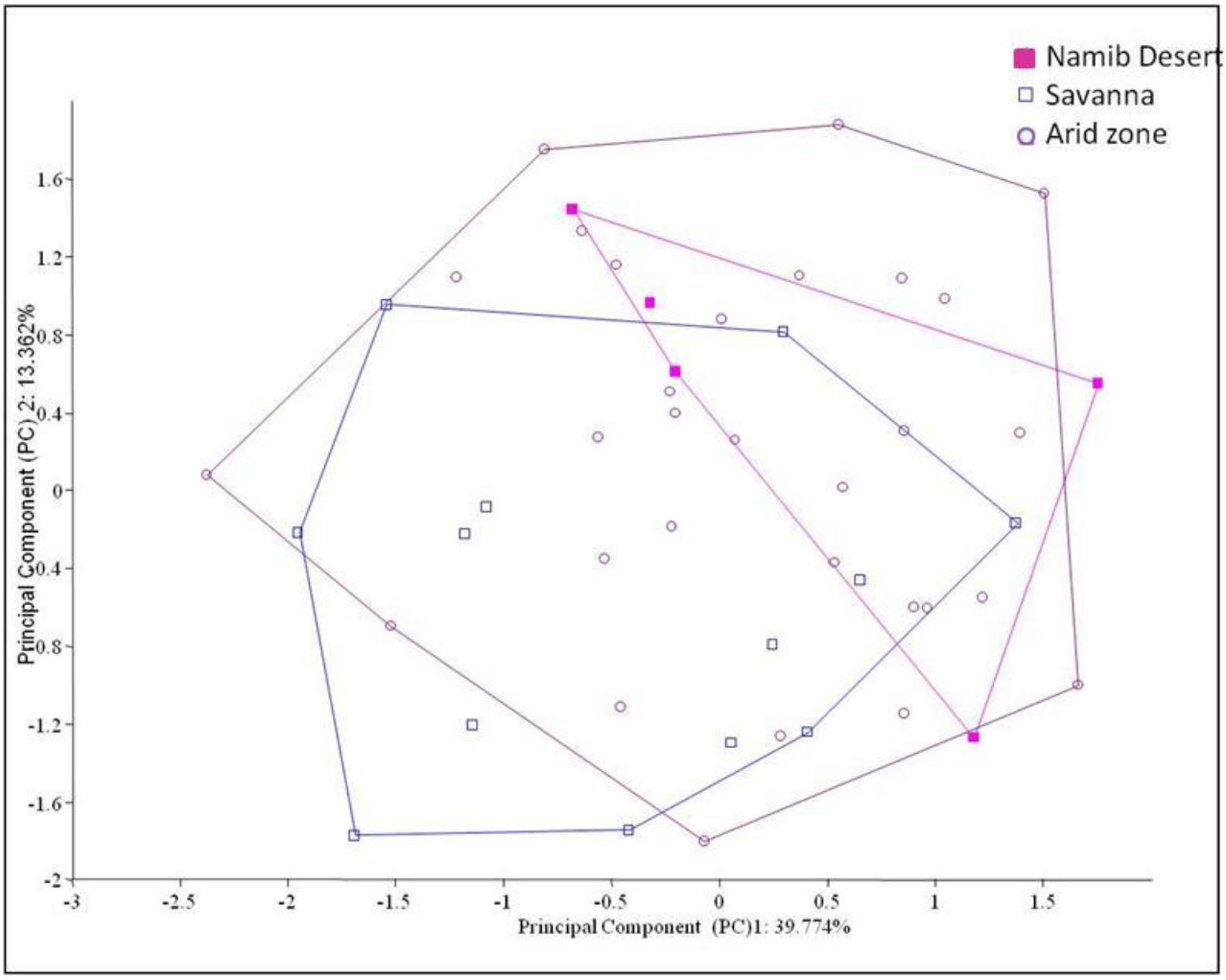


Figure 2.6: PCAscatter plot of the first two components (PC 1 and 2) run on 12 cranial measurements from 48 specimens of *G. paeba*. Symbols and colors identify individuals from different biotic zones.

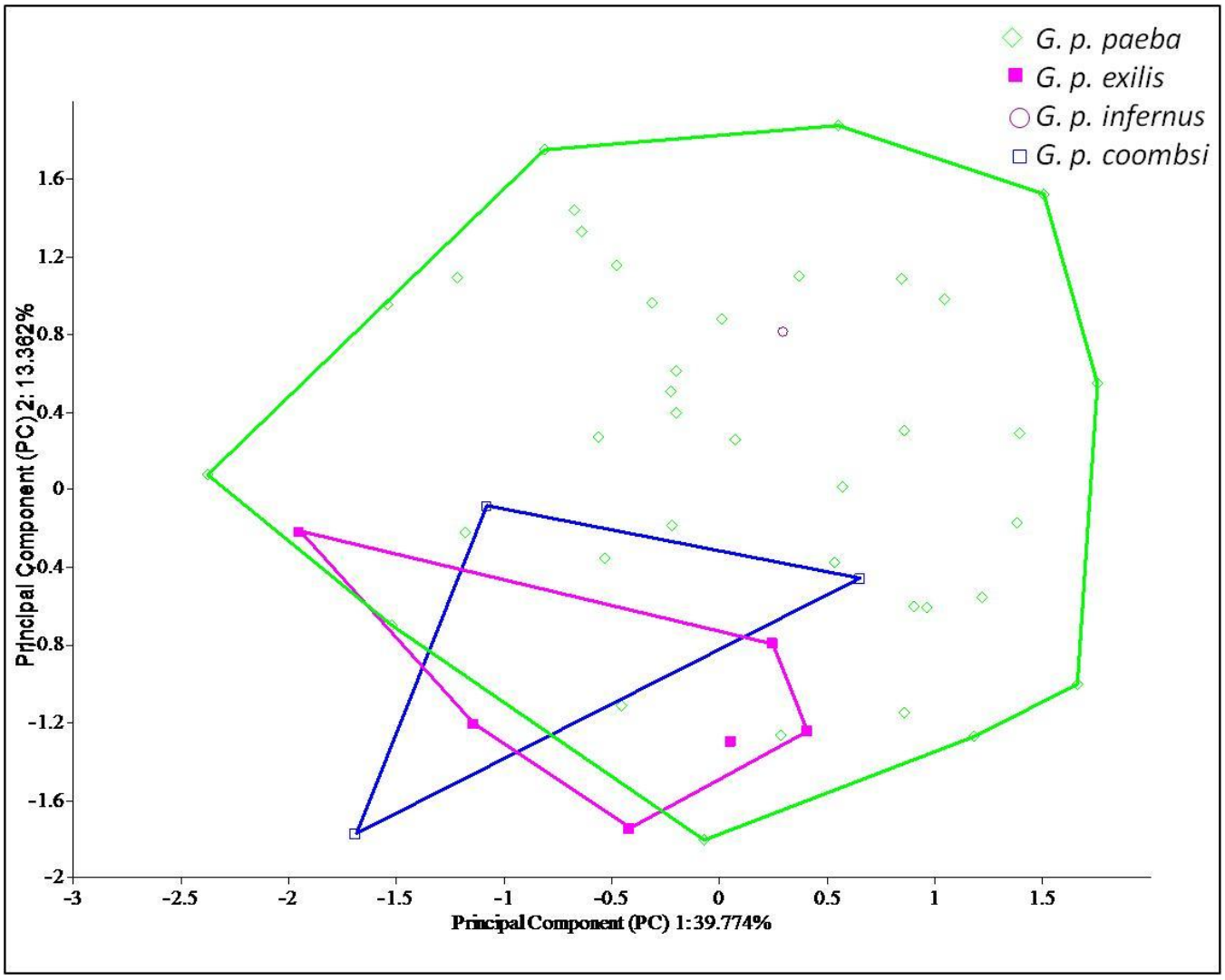


Figure 2.7: PCA scatter plot of the first two components (PC 1 and 2) run on 12 cranial measurements from 48 specimens of *G. paeba*. Symbols (colored) identify individuals from different localities representing subspecies.

Table 2.5: Component loadings of the first two principal components from principal component analysis of *G. paeba* with eigenvalue and total percent variation explained.

Variables	PC1	PC2
GLS	0.380748	-0.344511
BB	0.310024	0.143868
IB	0.282012	0.132156
RW	0.249038	0.009809
RH	0.273115	-0.323066
RL	0.347619	-0.199930
TBL	0.273656	-0.208897
UTR	0.294086	0.184270
BM[1]	0.195966	0.470563
OH	0.140272	0.517837
LMC	0.237151	0.347499
ML	0.382415	-0.103805
Eigenvalue	4.772830	1.603436
%variation explained	39.77359	13.36197

CVArevealed differentiation among specimens from some, but not all biotic zones (Fig. 2.8). For instance, specimens of *G. paeba* from the Savanna biotic zone grouped from -0.2 to the positive quadrant of CVA1 and from +0.2 to the negative quadrant of CVA2. Specimens from the Namib Desert do not overlap with individuals from the Savanna zone; they group on the negative quadrant of the x-axis and in the middle (±0.4) of the y-axis of the scatter plot. However, there is an overlap of individuals from the Savanna and South West Arid Zone (Fig. 2.8). The variations between OTUs were statistically non-significant (Wilks' Lambda: 0.452; $P< 0.15$). Character loadings differentiating specimens of Savanna biotic zone from other individuals of the Namib Desert were long upper tooth row, long rostral length, and broader breadth of the first molar (based on CVA character loadings, Table 2.7). Specimens from the Savanna zone were

represented by some individuals from north of the Soutpansberg which encompasses the region where *G. p. coombsi*occurs. However, when using *G. paeba* subspecies as OTUs, individuals that fall within the range of*G. p. coombsi* are scattered on the positive quadrant of the x-axis of the CVA scatter plot (Fig. 2.9). There is no overlap between individuals that represent *G. p. coombsi*and other subspecies. Although specimens within the range of *G. p. paeba* and *G. p. exilis* overlap, the former groups on the ± quadrant of both axes and the latter on the positive quadrant of CVA1 and 2 (Fig. 2.9). Variations between OTUs were statistically significant (Wilks' Lambda: 0.297; P= 0.0042). Character loadings differentiating *G. p. coombsi* from *G. p. paeba* and *G. p. exilis* were rostral height (60%) and mandibular length (43%) on the first CV, and great skull length (44%) and upper tooth row (38%) on the second CV (Table 2.8).

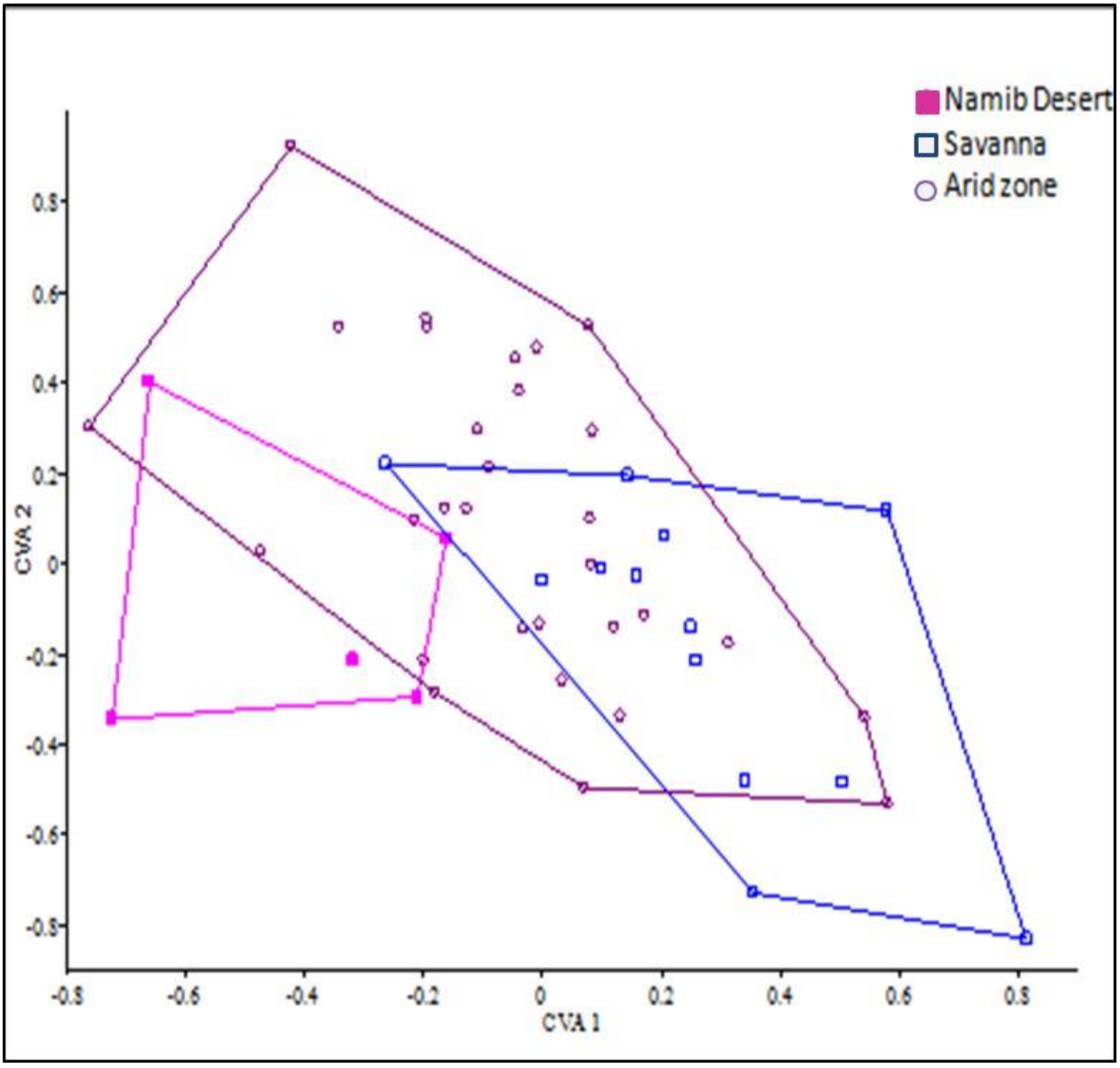


Figure 2.8: CVA scatter plot of the first two axes of discriminant functional analyses of 12 skull variables of seven Operational Taxonomic Units (OTUs) of *G. paeba.* Symbols represent OTUs.

Table 2.6: Character loadings of the first two canonical variates analysis of *G. paeba* when OTUs were grouped to biotic zones

Variables	CVA 1	CVA 2
GLS	0.22193	0.15123
BB	-0.20828	-0.00645
IB	-0.18616	-0.06387
RW	0.018636	0.41902
RH	0.32185	-0.30788
RL	-0.38622	0.11638
TBL	-0.34269	-0.30274
UTR	-0.50957	-0.23723
BM[1]	-0.37736	0.3229
OH	-0.22551	0.21113
LMC	0.16722	0.51548
ML	-0.14595	0.35583

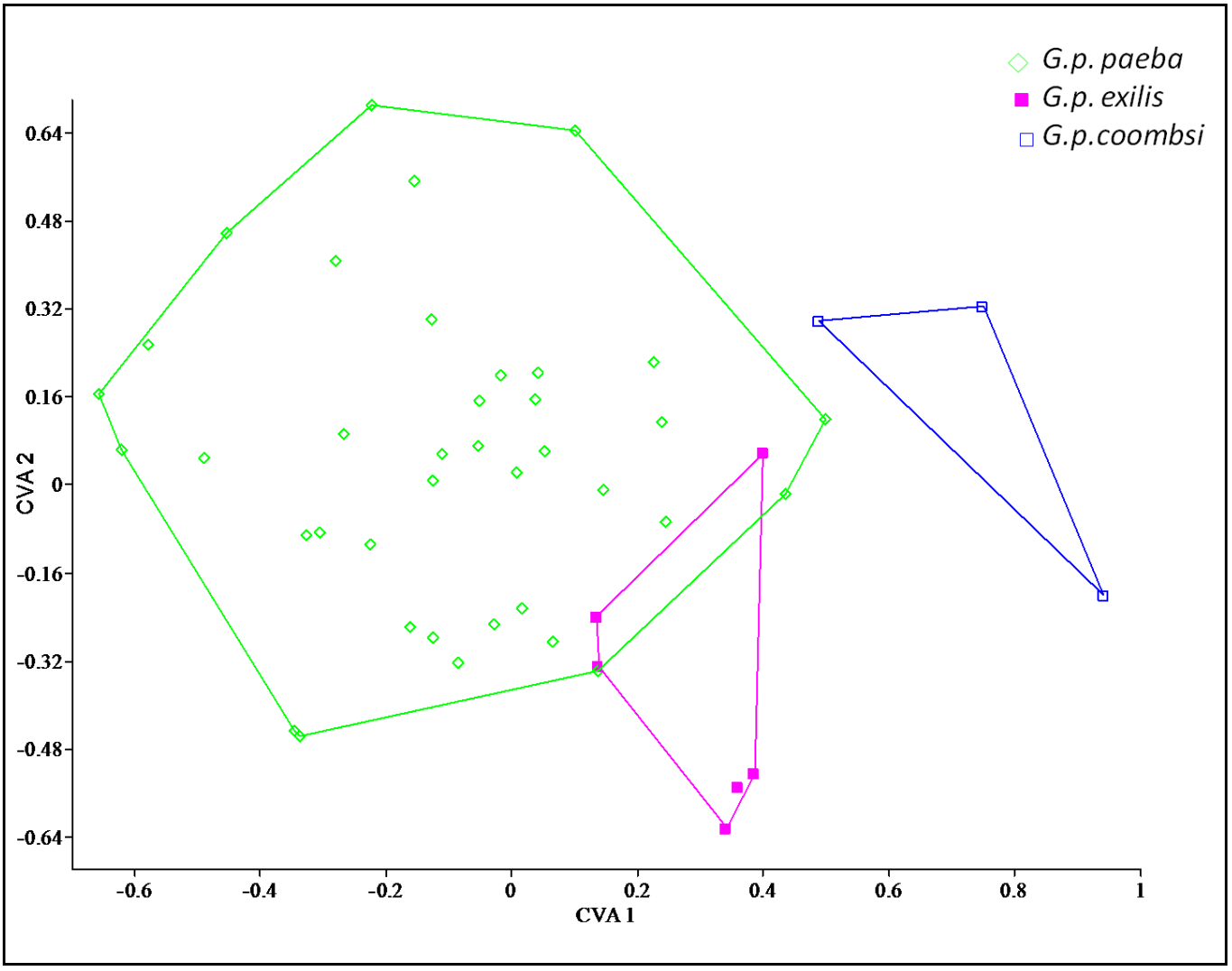


Figure 2.9: CVA scatter plot of the first two axes of discriminant functional analyses of 12 skull variables of three Operational Taxonomic Units (OTUs) of *G. paeba*. Symbols represent OTUs which represent individuals of the subspecies.

Table 2.7: Character loadings of the first two canonical variates analysis of *G. paeba* when OTUs were grouped according subspecies

Variables	CVA 1	CVA 2
GLS	0.003177	-0.44268
BB	-0.14377	-0.1882
IB	0.13919	0.34632
RW	-0.11017	-0.20281
RH	0.60764	0.38347
RL	0.058276	0.25499
TBL	-0.20205	-0.01872
UTR	-0.26813	0.38408
BM[1]	-0.375	0.26023
OH	-0.36088	0.30478
LMC	0.053099	0.26995
ML	-0.43451	0.11882

2.4 Discussion

2.4.1 The main findings

Desmodillus auricularis and *G. paeba* indicated a lack of cranial variation in relation to biotic zones and absence of sexual dimorphism but with significant age variation. Canonical variates analysis and principal component analysis retrieved no variation between and among biotic operational taxonomic units of *D. auricularis*. Although, these tests indicate that specimens from the Savanna Biotic Zone were slightly different from those emanating from Namib Desert, but both overlaps completely with South West Arid Zone individuals. However, all OTUs generally overlapped which confirms that *D. auricularis* is a monotypic species.

Multivariate results indicate that specimens of *G. paeba* from the Savanna biotic zone were different from individuals of the Namib Desert based on CVA but individuals from both these zones overlapped completely with South West Arid Zone individuals. Based on PCA, all individuals of the three biotic zones overlap with each other. Characters that differentiate Savanna individuals from the Namib Desert specimens were upper tooth row, rostral height and breadth of the first molar. However, when OTUs where grouped according to subspecies of *G. paeba*, CVA indicated that there is a variation between OTUs of subspecies. Individuals that represent *G. p. coombsi* do not overlap with either *G. p. paeba* or *G. p. exilis*, though there is a slight overlap of individuals representing *G. p. paeba* and *G. p. exilis*.

2.4.2 Subspecies delimitation of *G. paeba*

Univariate and PCA data results do not support the four subspecies of *G. paeba*which were previously recognised by Meester *et al*., (1986). However, CVA indicated that there is a variation between the OTU that represent *G. p. coombsi* and OTUs of *G. p. paeba* and *G. p. exilis* (Fig. 2.9) and the variation was significant (Wilks' lambda = 0.297; P= 0.004). Although only three individuals represent *G. p. coombsi*'s OTU, there is clear distinct of individuals of this OTU from the others based on CVA scatter plot (Fig. 2.9). However, this morphological variation may be a result of local adaptation. This is consistent with previously work which indicatethat species with wide geographic rangegenerally display geographical heterogeneity in attributes such as body sizes, morphology, food habits, and reproduction (Shine *et al*., 1998 and references therein).

Adequate sampling of this subspecies may reveal the extent of variation suggested by these three specimens.

The subspecies *G. p. infernus* is not represented in canonical variates analysis because a single specimenwas sampled. However, on principal components analysis, *G. p. infernus* individual group with*G. p. paeba* individuals and does not overlap with individuals that represent *G. p. coombsi* and *G. p. exilis*- the latter completely overlap with each other. Specimens of G. *p. coombsi*are distributed within the Southern Savanna Woodlands biotic zone and of *G. p. exilis* within the Southern Savanna Grassland (Smithers, 1983). These subspecies are differentiated by character loadings; *G. p. coombsi* has a taller rostrum and shorter mandible than *G.p.exilis* and *G. p.paeba*. Albeit there is some overlap *G. p. coombsi* also tend to have longer upper toothrows and shorter skull lengths than *G.p.exilis* (CVA2 character loadings, Table 2.7).

In view of previous subspecies demarcation and results obtained in this study, *G. paeba* appears to be comprised of two subspecies or operational taxonomic units; *G. p. paeba* (South West Arid Zone) and*G. p. coombsi* (Southern Savanna woodlands). Simple diagnostic ratios from standard statistics suggest thatrostral height, skull length, and mandibular length (Table 2.7) may be useful in separating subspecies.

2.5 Conclusions

The morphological data used in this study confirm that there is no sexual dimorphism in either of the species studied. Further, the three main biotic subdivisions of southern Africa where the study animals occur are not reflected in the morphometrics analysis of either species. Further, geographic fixtures of southern African subregion, especially the effect of Orange River as the barrier observed on other southern African dwelling species is not evident in the morphometric variation (or lack thereof) of *D. auricularis* and *G. paeba*.

Morphometric analyses indicate that there is little or no geographic variation within *D. auricularis* –this is most likely an artefact of the low sample sizes. In contrast, this analysis suggests the four recognised subspecies of *G. paeba*may be reduced to two: *G. p. paeba* which has a widest distribution and consist of specimens from the South West Arid Zone, and *G. p. coombsi* comprise individuals representing the Southern Savanna

Woodlands.Nonetheless,variables that can be used to distinguish these subspecies are rostral height and mandibular length. Further analysis could be done including more variables and geometric morphometrics analysis to assess more specifically what shape changes may be occurring within each of the species across their distribution. Finally, the results of this analysis suggest that only one lineage exist in *D. auricularis* while *G. paeba* may contain multiple lineages.

Chapter 3

Comparative phylogeography of *Desmodillus auricularis* and *Gerbillurus paeba*

3.1 Introduction

Phylogeography overlays the phylogenetic information of taxa onto their geographic distributions in an attempt to identify the processes that drive the spatial partitioning of genetic variation (Avise, 2000). Phylogeographic patterns can then be interpreted in the context of the life history structure of a species including aspects such as the social structure, demographic dynamics as well as migration patterns (gene flow). For instance, social species might be expected to have less genetic structure because there is high gene flow between them than solitary species, all other things being equal. The genetic structure of a species can also be shaped by habitat preference; specifically, species occupying a more continuous habitat might be characterized by a panmictic structure or isolation-by-distance (depending on its vagility) whereas species occupying disjunct habitats may have less gene flow between demes (see e.g. Smit *et al.,* 2010). Life history structure of a species can be used to determine observed patterns of genetic diversity while phylogeography and landscape characteristics can be used in understanding local adaptation and the processes and patterns of gene flow (Mora *et al.,* 2006). In this chapter, I will explore effects that habitat and life history characteristics may have on the genetic structure of species using two sympatrically distributed gerbil species, *D. auricularis* and *G. paeba*, as exemplars.

Molecular markers used to investigate the genetic structure across species' distributions should have a traceable genealogy (Avise, 2000). In this study, mitochondrial DNA (mtDNA) was used because of several attributes. These include a relatively fast rate of evolution, a small effective copy number which captures evolutionary history easily (because it is haploid and maternally transmitted) and absence of recombination (Brown *et al*., 1979; Lansman *et al*., 1983; Avise *et al*., 1989; Avise, 1994). Notwithstanding these attributes, the genes and gene order are relatively

conserved and can be amplified using universal primers (Irwin *et al.*, 1981). As a result, mtDNA has been used widely in phylogeographic studies of South African small mammals including Smith's red rock rabbit, *Pronolagus rupestris* (Matthee and Robinson, 1996), several elephant-shrew species (including *Elephantulus edwardii*, Smit *et al.*, 2007; *Elephantulus rupestris* and *Macroscelides proboscideus*, Smit *et al*., 2010),the four-striped mouse, *Rhabdomyspumilio* (Rambau *et al.*, 2003), Namaqua rock mouse, *Micaelamys namaquensis* (Russo *et al.*, 2010) and the Karoo Bush Rat,*Myotomysunisulcatus*(Edwards *et al*., 2011).

Several physical attributes, which may have a profound influence on the genetic structure of small mammal taxa, characterize the south-western part of southern Africa. These include the Great Escarpment, the Cape Fold Mountains, large river systems such as the Orange River and the heterogeneous landscape. Specifically, the Great Escarpment has been shown to structure genetic variation in the Karoo Bush Rat(Edwards *et al*., 2011). Further, genetically distinct groups in the Cape rock elephant-shrew (Smit *et al.*, 2010) as well as Cape dwarf chameleon (Tolley *et al.*, 2004) have been correlated to different mountain regions. The Orange River, which forms the border between Namibia and South Africa, is the largest river in southern Africa and significantly influences movement of taxa such as western rock elephant-shrew (Smit *et al.*, 2010) and also forms the northern boundary of other taxa such as the Cape rock elephant-shrew (Smit *et al.*, 2007). It has also been hypothesised that intermittent flow of the Orange River disrupted gene flow in the Rock agama (Matthee and Flemming, 2002). Interestingly, this phenomenon does not appear to have an effect on *M. proboscideus* (Smit *et al*., 2010), suggesting that the impact of the Orange River as a barrier may be different between species.

The preferred habitat of the two gerbil species included in this study appears similar at the coarse scale. However, they differ markedly in their micro-habitat preference (see Skinner and Chimimba, 2005). *Desmodillus auricularis* prefer hard ground with adequate grass and bush cover. They are associated with the fringes of pans (in the Kalahari) or gravel plains (Namibia) and are almost never found in sand dunes. In contrast, *G. paeba* prefer sandy soils with some cover but avoid areas with high plant species richness. They are common on Kalahari sands but are absent from harder ground. The effect that these different habits and habitats have on the genetic structure of these two gerbil species has not yet been assessed. In addition, whether the

current taxonomy accurately reflects genetic variation (four subspecies are recognised in *G. paeba* while *D. auricularis* is monotypic), or alternatively, whether a taxonomic revision may be warranted will be assessed.

The aims of this section of the project are twofold:

1. To describe and compare the spatial distribution of genetic variation of *G. paeba* and *D.auricularis* across their ranges.
2. To ascertain whether the currently recognized four subspecies of *G. paeba* have a genetic basis, i.e. to what extent are they genetically divergent?

3.2. Materials and Methods

3.2.1. Specimens

Tissue samples were taken from skin and / or skull of specimens (N=250) held at the Ditsong Museum of Natural History (former Transvaal Museum, Pretoria), Durban Natural Science Museum and the National Museum (Bloemfontein). These museum tissue samples represent a collecting period spanning more than 80 years, the oldest specimen having been collected in the 1920s. Tissue samples were taken using sterile blades from a subset representative of the distribution of the species. For *G. paeba* specifically, the emphasis was on or near localities where the different subspecies occur, i.e. Waterpoort Soutpansberg in the Limpopo Province (*G. p. coombsi*), Alexandria district in the Eastern Cape (*G. p. exilis*) and the Skeleton coast, northern Namibia (*G. p. infernus*). Museum material was supplemented by fresh materials (trapped using Sherman™ traps and Supa-Kill Rat and mouse traps™) from Sutherland in the Northern Cape Province. Traps were baited with peanut butter and oats; they were set in the afternoon and checked early in the morning and late afternoon to ensure animals did not die from exposure to the elements.

3.2.2.DNA extraction[4]

DNA was extracted from skin and skull tissue samples using the DNeasy Blood & Tissue Kit or the DNA-Micro Kit (Qiagen) following the manufacture's protocols. Briefly, tissue was incubated at 55ºC in 180µl ATL buffer and 20 µl proteinase K for about 3 hours or until the tissue was completely lysed. Following tissue lyses, 200 µl buffer AL was added to the sample, vortexed for 15 seconds and incubated at 70ºC for 10 minutes. After incubation, 200 µl of ethanol (96 %) was added to the sample and mixed thoroughly and when using DNA-Micro Kit, samples were incubated for 5 minutes at room temperature. The sample solution was transferred onto 2ml spin columns and centrifuged at 8000 rpm for 1 minute. The column was washed with 500 µl buffer AW1 and centrifuged at 8000 rpm for 1 minute, and washed again with 500 µl buffer AW2 and lastly centrifuged at 13000 rpm for 3 minutes to remove residual buffers containing ethanol which might inhibit DNA amplification. To maximize the yield; DNA was eluted twice with buffer AE; first with 30 µl after incubated at room temperature for 20 min, secondly with 20µl after incubated at room temperature for 15 min and centrifuged at 13000 rpm for 1 minute.

3.2.3. Polymerase chain reaction (PCR), primers and sequencing

A segment of the mitochondrial cytochrome *b* gene (400 base pairs) was amplified using primers tRNA-GluA: 5´-TGACTTGAARAACCAYCGTTG-3´ and tRNA-GluJ: 5´-CCC TCAGAATGATATTTGTCCTCA-3´ (Palumbi *et al*., 1991). PCR reactions were carried out using a GeneAmp® PCR system 2700 thermal cycler (Applied Biosystems) using the following regime: initial denaturation at 94 ºC for 4 minutes followed by 36 cycles comprised of denaturation for 45 seconds at 94 ºC, annealing for 45 seconds at 48 ºC, and extension for 35 seconds at 72 ºC. The reaction mixture (50 µl) contained 21.8 µl dH_2O, 5 µl Buffer, 10 µl primers, 100 mM dNTPs, 10 mM $MgCL_2$, 0.2 µl Ampli*Taq*® (Southern Cross) polymerase and 5 µl DNA.

[4]Initially, DNA was extracted from 250 specimens (*D. auricularis* and *G. paeba*), of these only 26 specimens of *G. paeba* from 12 localities and 41 *D. auricularis* specimens representing 25 localities were successfully sequenced and analysed (Table 3.1 and 3.2; Fig. 2.a and b).

PCR products were electrophoresed in 1% agarose gels. Bands were excised with a sterile surgical blade and purified with a commercial kit (Illustra™, GE Healthcare). BigDye Chemistry was used to perform cycle sequencing and the products were run on an automated sequencer (AB 3100, Applied Biosystems). Once obtained, all sequences were first screened using BLAST searches against sequences archived in public data bases such as GenBank**,** and then edited and aligned using BioEdit version 7.0.5 (Hall, 2005).

Table 3.1: List of *D. auricularis* (N=41) specimens from which sequences were successfully obtained. For each specimen the following data were captured: animal identity numbers, museum identity numbers, locality names, country (province), GPS coordinates, sex, collection date, locality numbers and mtDNA haplotype numbers.

Animal ID	Museum ID	Locality name	Country (Province)	GPS Coordinates	Gender	Collection date	Locality number	MtDNA Haplotype
Desmodillus1	NMB 6585	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	M	02-Aug-88	1	1
*Desmodillus*2	NMB 6586	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	M	02-Aug-88	1	1
*Desmodillus*3	NMB 6588	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	F	02-Aug-88	1	1
*Desmodillus*4	NMB 6584	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	M	02-Aug-88	1	1
*Desmodillus*5	NMB 7785	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	M	02-Aug-88	1	1
*Desmodillus*6	NMB 1299	Elandsbay	South Africa (Western Cape)	32° 18′ 45″S 18° 21′ 00"E	F	14-Feb-39	24	1
*Desmodillus*7	NMB 1300	Elandsbay	South Africa (Western Cape)	32° 18′ 45″S 18° 21′ 00"E		14-Feb-39	24	2
*Desmodillus*8	NBM 6587	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	F	02-Aug-88	1	1
*Desmodillus*9	NMB 7783	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	F	02-Aug-88	1	1
*Desmodillus*10	NMB 7782	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	M	02-Aug-88	1	1
*Desmodillus*11	NMB 7784	Bainsvlei	South Africa (Free State)	29° 12′ 45″S 26° 08′ 30"E	F	02-Aug-88	1	1
*Desmodillus*12	NMB 1330	Rietput	South Africa (Free State)	29° 07′ 30″S 24° 37′ 30"E	F	27-Feb-72	2	1
*Desmodillus*13	NMB 1482	Middelsbron	South Africa (Free State)	30° 15′ 28″S 25° 16′ 14"E	M	09-Aug-72	3	1
*Desmodillus*14	NMB 1301	Elandsbay	South Africa (Western Cape)	32° 18′ 45″S 18° 21′ 00"E	M	14-Feb-39	24	1
*Desmodillus*15	NMB 1302	Elandsbay	South Africa (Western Cape)	32° 18′ 45″S 18° 21′ 00"E		26-Jan-39	24	1
*Desmodillus*16	NMB 1484	Middelsbron	South Africa (Free State)	30° 15′ 28″S 25° 16′ 14"E	M	08-Aug-72	3	1
*Desmodillus*17	NMB 1483	Middelsbron	South Africa (Free State)	30° 15′ 28″S 25° 16′ 14"E	F	07-Aug-97	4	3
*Desmodillus*18	NMB 1859	Kleinfontein	South Africa (Free State)	30° 25′ 52″S 26° 03′ 04"E	M	03-Mar-75	5	3
*Desmodillus*19	NMB 11232	Kareepoort	South Africa (Free State)	30° 14′ 45″S 25° 05′ 09"E	F	10-Jan-97	4	3
*Desmodillus*20	NMB 11426	Sandymount park	South Africa (Free State)	29° 44′ 52″S 25° 18′ 59"E	F	17-Jul-98	6	4
*Desmodillus*21	NMB 11324	Sandymount park	South Africa (Free State)	29° 44′ 52″S 25° 18′ 59"E	M	05-May-98	6	4
*Desmodillus*22	TM 64	kururman	South Africa (Northern Cape)	27° 27′ 00″S 23° 25′ 59"E	F	29-May-04	7	1
*Desmodillus*23	DMNUM 266	Bedford	South Africa (Eastern Cape)	32° 40′ 58″S 26° 06′ 12"E	M	26-Jun-62	8	1
*Desmodillus*24	TM 7620	Ohikonda	Namibia	18° 54′ 33″S 16° 58′ 42"E	M	30-Feb-32	9	1
*Desmodillus*25	TM 8086	Klipfontein	South Africa (Northern Cape)	29° 52′ 30″S 17° 52′ 30"E	M	19-Aug-37	18	3
*Desmodillus*26	TM 15138	Palmenhorst	Namibia	22° 41′ 37″S 14° 54′ 00"E	M	26-Oct-65	10	1
*Desmodillus*27	TM 22972	4 MI Nwaus	Namibia	26° 22′ 30″S 16° 37′ 30"E	F	12-Jan-58	11	1
*Desmodillus*28	TM 23045	Namibia		22° 52′ 30″S 17° 52′ 30"E	F	18-Feb-50	12	1
*Desmodillus*29	TM 10976	Sesfontein	Namibia	19° 07′ 30″S 13° 37′ 30"E	F	07-Jul-51	13	1
*Desmodillus*30	TM 5082	Klaver	South Africa (Western Cape)	31° 46′ 59″S 18° 37′ 00"E	F	06-Nov-26	20	1

Table 3.1: (continued)

Animal ID	Museum ID	Locality name	Country (Province)	GPS Coordinates	Gender	Collection date	Locality number	MtDNA Haplotype
*Desmodillus*31	TM 43634	E,Sendlingsdrift 10 KM	South Africa (Northern Cape)	28° 07′ 36″S 16° 59′ 13″E	M	30-Sep-92	14	1
*Desmodillus*32	TM 4330	Swakopmund	Namibia	22° 40′ 22″S 14° 31′ 39″E	M	20-Feb-25	15	1
*Desmodillus*33	TM 5080	Zak River	South Africa (Northern Cape)	30° 52′ 30″S 20° 22′ 30"E	M	24-Jul-26	16	1
*Desmodillus*34	TM 5077	Calvinia	South Africa (Northern Cape)	31° 22′ 30″S 19° 52′ 30"E	F	14-Aug-26	17	1
*Desmodillus*35	TM 8088	Ookiep	South Africa (Northern Cape)	29° 52′ 30″S 17° 52′ 30"E	F	24-Aug-37	18	1
*Desmodillus*36	TM 12878	Karasburg	Namibia	27° 22′ 30″S 18° 37′ 30"E	M	09-Sep-49	19	1
*Desmodillus*37	TM 5073	Atties	South Africa (Western Cape)	31° 46′ 59″S 18° 37′ 00"E	M	19-Jul-27	20	1
*Desmodillus*38	TM 7624	Ongandjera	Namibia	18° 07′ 30″S 14° 37′ 30"E	M	00-Jul-32	21	1
*Desmodillus*39	TM 8797	Eendekuil	South Africa (Western Cape)	32° 41′ 17″S 18° 53′ 02"E	F	28-Aug-37	22	1
*Desmodillus*40	TM 22920	Kariega Station	South Africa (Eastern Cape)	33° 23′ 24″S 25° 26′ 22"E	F	22-May-26	23	5
*Desmodillus*41	TM 23050	Kariega Station	South Africa (Eastern Cape)	33° 23′ 24″S 25° 26′ 22"E	M	16-Oct-63	23	5

Table 3.2: List of *G. paeba* specimens (N=26) from which sequences were successfully obtained. For each species the following data were captured: animal identity numbers, museum identity numbers, locality names, country (province), GPS coordinates, sex, collection date, locality numbers and mtDNA haplotype numbers.

Animal ID	Museum ID	Locality Name	Country (Province)	GPS Coordinates	Gender	Collection date	Locality number	MtDNA Haplotype
Gerb1	NMB 11904	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′ 56"E	M	14-Apr-00	5	5
Gerb2	NMB 11937	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′56"E	F	14-Apr-00	5	5
Gerb3	NMB 11886	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′56"E	F	12-Apr-00	5	5
Gerb4	NMB 11892	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′56"E	F	13-Apr-00	5	5
Gerb5	NMB 11896	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′56"E	M	12-Apr-00	5	10
Gerb6	NMB 11898	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′56"E	M	12-Apr-00	5	5
Gerb7	NMB 2908	Driehoek	South Africa (Free State)	29° 07′ 30″S 24° 37′ 30″E	M	03-Oct-79	1	2
Gerb8	NMB 8687	Zoutpansdrift	South Africa (Free State)	29° 45′ 04″S 24° 47′ 09"E	F	26-Jan-93	10	4
Gerb9	NMB 11925	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′ 56"E	F	11-Apr-00	5	5
Gerb10	NMB 2911	Driehoek	South Africa (Free State)	29° 07′ 30″S 24° 37′ 30″E	F	03-Oct-79	1	2
Gerb11	NMB 8690	Zoutpansdrift	South Africa (Free State)	29° 45′ 04″S 24° 47′ 09"E	M	26-Jan-93	10	3
Gerb12	NMB 11893	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′ 56"E	M	13-Apr-00	5	5
Gerb13	TM 6895	Upington, 76mi N	South Africa (Northern Cape)	27° 52′ 30″S 20° 21′ 30″E	M	02-Jul-32	4	6
Gerb14	TM 37408	Dikgatlong_Deben rd, 25.7 MI	South Africa (Northern Cape)	27° 22′ 30″S 22° 52′ 30"E	M	17-Aug-50	3	8
Gerb15	TM 27444	Augrabies Waterfall Nat. Park	South Africa (Northern Cape)	28° 37′ 30″S 20° 22′ 30"E	F	07-May-77	2	8
Gerb16	TM 27429	Augrabies Waterfall Nat. Park	South Africa (Northern Cape)	28° 37′ 30″S 20° 22′ 30"E	M	06-May-77	2	9
Gerb17	NMB 11914	Sandveld Nature Reserve	South Africa (Free State)	27° 49′ 58″S 25° 54′ 56"E	F	14-Apr-00	5	7
Gerb18	SLGp 5 (fresh)	Sutherland	South Africa (Northern Cape)	32° 24′ 29.1″S 20°54′ 48.6″E	?	15-Dec-09	6	6
Gerb19	SLGp 9 (fresh)	Sutherland	South Africa (Northern Cape)	32° 24′ 29.1″S 20°54′ 48.6″E	M	16-Dec-09	6	6
Gerb20	SGLp 2 (fresh)	Sutherland	South Africa (Northern Cape)	32° 24′ 29.1″S 20°54′ 48.6″E	F	14-Dec-09	6	6
Gerb21	TM 28850	W Gobabeb 10 km	Namibia	23° 33′ 35″S 15° 02′ 25″E	F	24-May-78	8	6
Gerb22	TM 10986	Seeheim, fish river	Namibia	26° 52′ 30″S 17° 52′ 30″E	M	23-Jul-51	7	6
Gerb23	TM 33184	Namibia	Namibia	23° 07′ 30″S 14° 37′ 30″E	F	12-Jul-80	9	6
Gerb24	TM 37331	Sunday River mouth,	South Africa (Eastern Cape)	33° 37′ 30″S 25° 52′ 30″E	M	18-Oct-59	11	1
Gerb25	TM 37363	Farm Sudbury	South Africa (Limpopo Province)	29° 22′ 30″S 22° 52′ 30″E	F	05-Jun-69	12	5
Gerb26	TM 37329	Sunday River mouth,	South Africa (Eastern Cape)	33° 37′ 30″S 25° 52′ 30″E	M	18-Oct-59	11	11

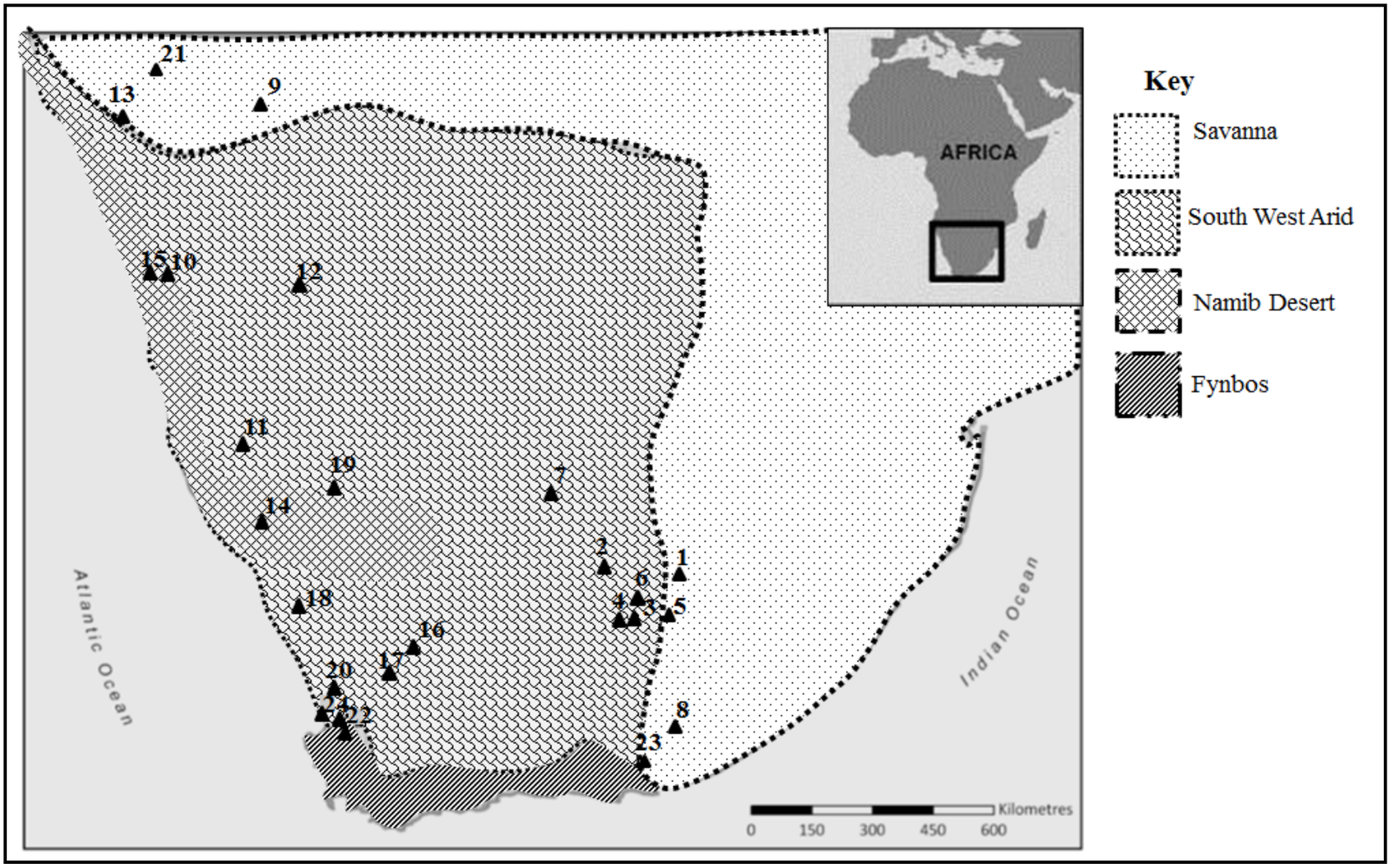


Figure 3.1A: Southern Africa map with localities of *D. auricularis* specimens used in this study. Numbers associated with the localities link to further information about the localities and the specimens in Table 3.1. The boundaries of the biotic zones are indicated by different shadings.

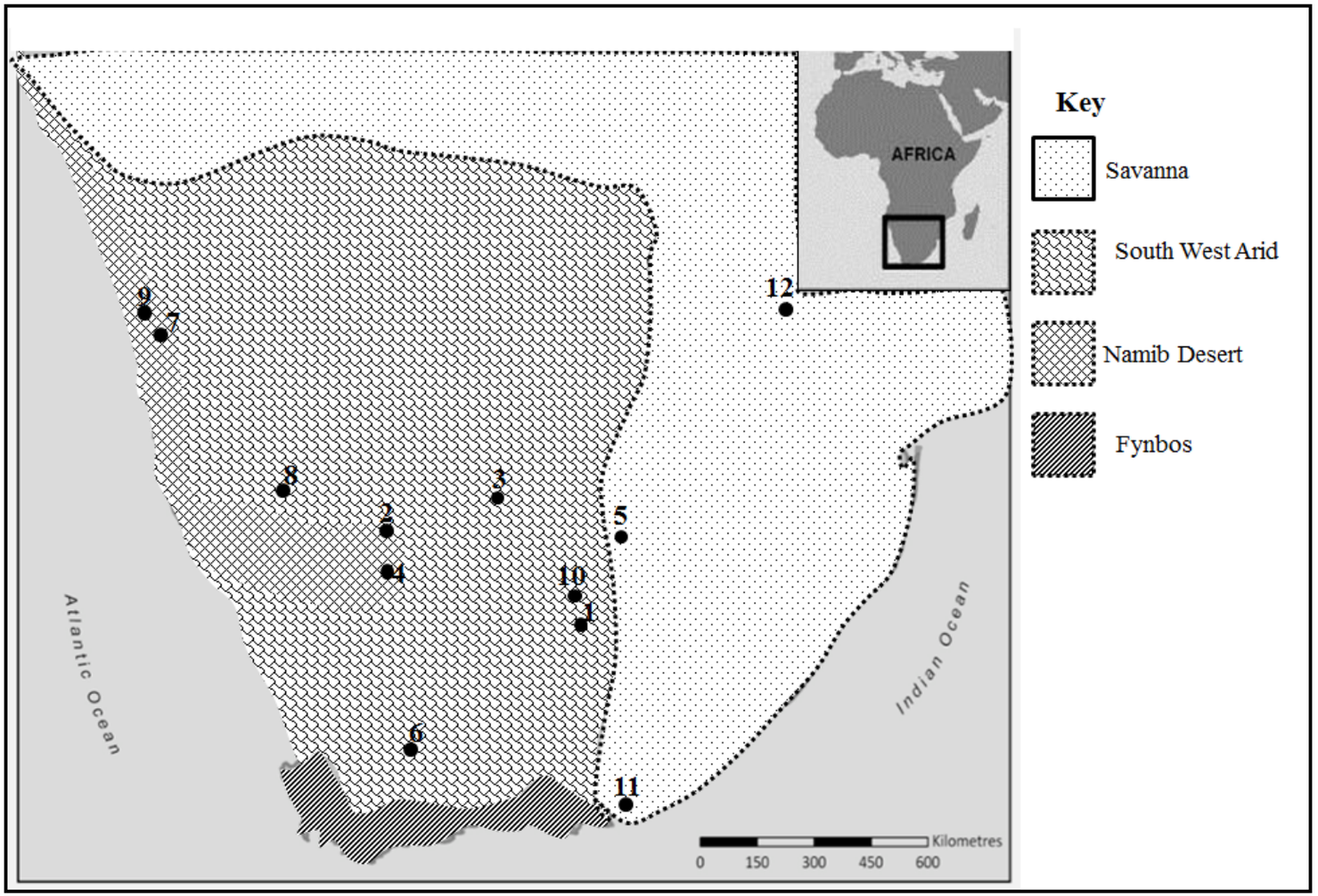


Figure 3.1B Southern Africa map with localities of *G. paeba* specimens used in this study. Numbers associated with the localities link to further information about the localities and the specimens in Table 3.2. The boundaries of the biotic zones are indicated by different shadings.

3.2.4. Population genetic analysis

Nucleotide diversity (π) and haplotype diversity (h) were estimated using DnaSp version 4.10.9 (Rozas *et al.,* 2003). A haplotype network was constructed using TCS version 1.2.1 (Clement *et al.*, 2000) and redrawn by hand. Mantel tests were used to correlate geographic distance against genetic distance in order to assess isolation by distance (Rousset, 1997). Significance was determined through random permutations.

3.2.5. Phylogenetic analysis

jMODELTEST ver. 0.1.1 was used to determine the mode of nucleotide substitutions and the optimal model of evolution for the respective data sets (Posada, 2008). The Akaike information criterion (AIC), which optimizes the number of criteria describing the data, was chosen (Akaike, 1973).

Sequences were analysed using neighbour joining (NJ) and maximum parsimony (MP) implemented in PAUP*4.0b10. The heuristic search option was selected with tree bisection and reconnection (TBR) branch swapping algorithm using 100 random taxon stepwise additions. For the neighbour joining analyses, the optimal model was selected. Nodal support was determined through 1000 parametric bootstraps.

Bayesian trees were constructed in MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). Ten Monte Carlo Markov chains were run for 60,000 generations for *G. paeba* and 50,000 generations for *D. auricularis*, sampling every 1000th generation. The first 10% of the trees were discarded as burn-in, and posterior probabilities for the nodes were derived from the remaining trees.

3.2.6. Outgroup selection

Sequence data of *Desmodillus* were polarised using *G. paeba* (GenBank accession number AJ430557) and *G. brantsii* (GenBank accession number AM409393) based on their phylogenetic placement as sister taxa to *Desmodillus* (Pavlinov *et al*., 1990; Chevret and Dobigny, 2003). Similarly, sequence data of *Gerbillurus* was polarised using congerics, *G. setzeri* (GenBank accession number AJ430558) and *G. brantsii* (GenBank accession number AM409393) which

were shown to be sister taxa based on molecular and morphological data (Pavlinov *et al*., 1990; Chevret and Dobigny, 2005; Colangelo *et al*., 2007).

3.3. Results

3.3.1. Population structure of *D. auricularis*

Forty one specimens representing 24 localities were successfully amplified and sequenced (Table 3.1, Fig. 3.1A). The fragments contained 394 base pairs of partial mtDNA cyt *b* gene with base frequencies typical of the cyt *b* gene: A = 29.41 %, C =28.20 %, G = 13.71 % and T = 28.69 %. Excluding the outgroups, 291 characters of the 394 bp were constant, 57 were variable, and 49 variable characters were parsimony informative.

Five haplotypes characterized the 41 specimens. The most common haplotype (H1), which is present in 38 specimens, is separated from other haplotypes by between six and seven mutational steps (Fig 3.2). The common haplotype is distributed almost all over the distribution of the species (Fig. 3.3). Haplotype diversity is low reflecting the large number of specimens sharing the common haplotype (H1). Nucleotide diversity (π) is 0.00654 ± 0.00397. A negative relationship was found between geographic and F_{ST} values, this was weak and non-significant ($r = 0.013$, $p = 0.41$, see Fig. 3.4) indicating no isolation by distance.

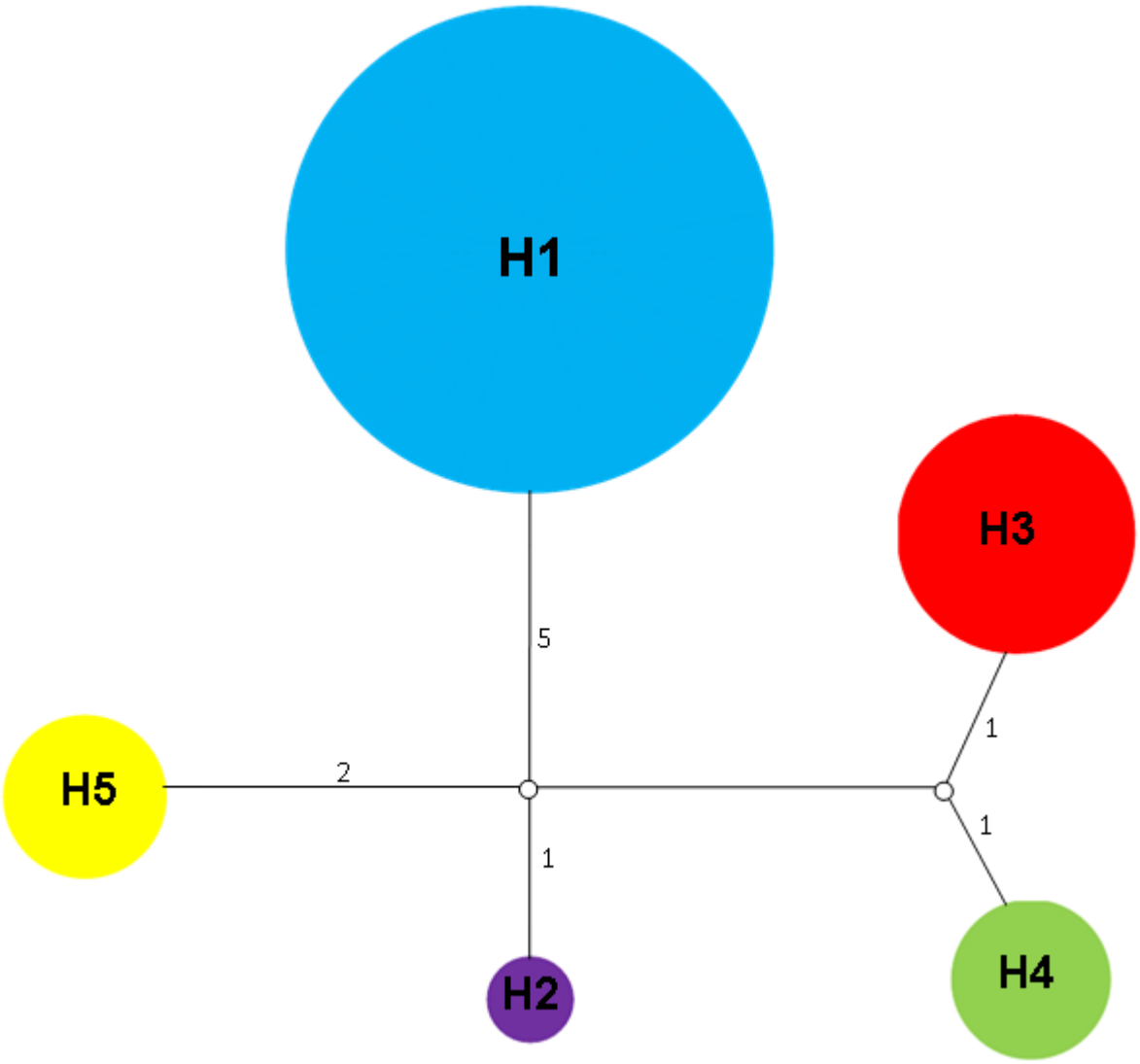


Figure 3.2: The haplotype network of *D. auricularis* derived from 394bp of mtDNA cyt *b* sequences for 41 specimens representing 24 localities. H1-H5 are the five haplotypes (connected at 95 % confidence limits) and the size of the circle is proportional to the number of individuals sharing that haplotype. Small circles indicate inferred or un-sampled haplotypes and the numbers of mutational steps separating haplotypes are indicated as numerical values.

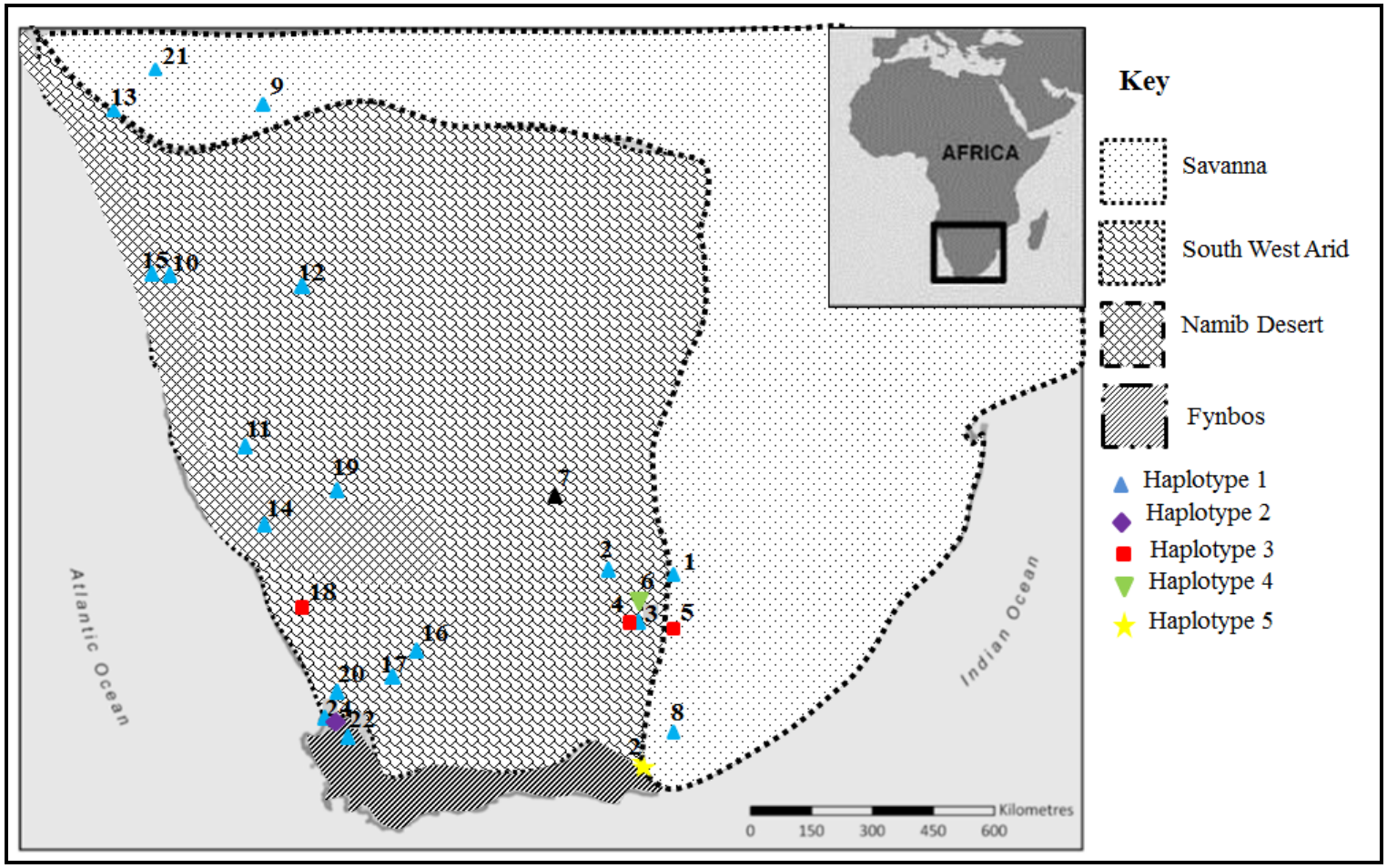


Figure 3.3: Geographic distribution ofhaplotypes of *D. auricularis* derived from 394bp of mtDNA cyt *b* sequences for 41 specimens representing 24 localities in the major biotic zones of southern Africa. Colours of haplotypes are proportional to the colours of haplotype in figure 3.2 and are represented by different shapes. Numbers correspond with localities in table 3.1.

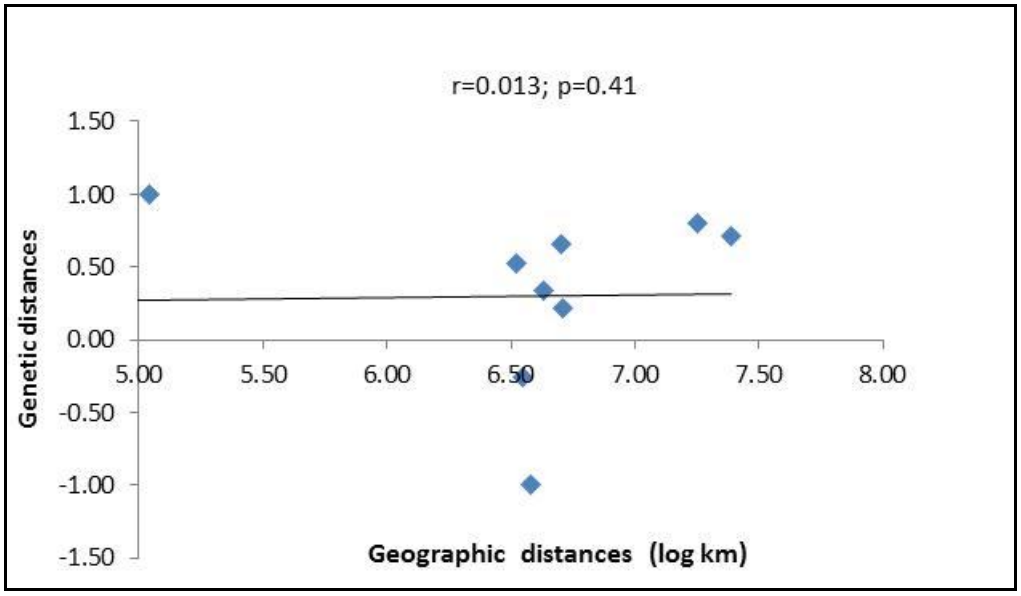


Figure 3.4: Scatter plot of geographic distances (log transformed km) against genetic distances. Significance was determined through 1000 replicates.

3.3.2. Phylogenetic analysis of *D. auricularis*

JMODELTEST (jModeltest 0.1.1) selected HKY as the optimal model of evolution. Maximum parsimony, neighbour joining and Bayesian inference retrieved nearly identical tree topologies comprised of well supported two clades (Fig. 3.5). Uncorrected sequence divergences separating these two clades are not large and ranged between 1.52 - 1.77%. The first clade (clade A) is comprised of four haplotypes (H2, H3, H4 and H5) with five individuals representing Free State Province; two from Eastern Cape Province and one each from Northern Cape Province and the Western Cape Province and is distributed on the western side of southern Africa (see Table 3.1). Uncorrected sequence divergence within this clade ranged from 0.51-1.02% (Table 3.3). Clade B is comprised of only one haplotype (H1, N=38) which is distributed across Namibia, the Free State Province, Northern Cape Province, Western Cape Province and Eastern Cape Province (Fig. 3.5) and represents almost the entire range of the species (see Table 3.1). However, both clades overlap within the western biotic zones of the subregion.

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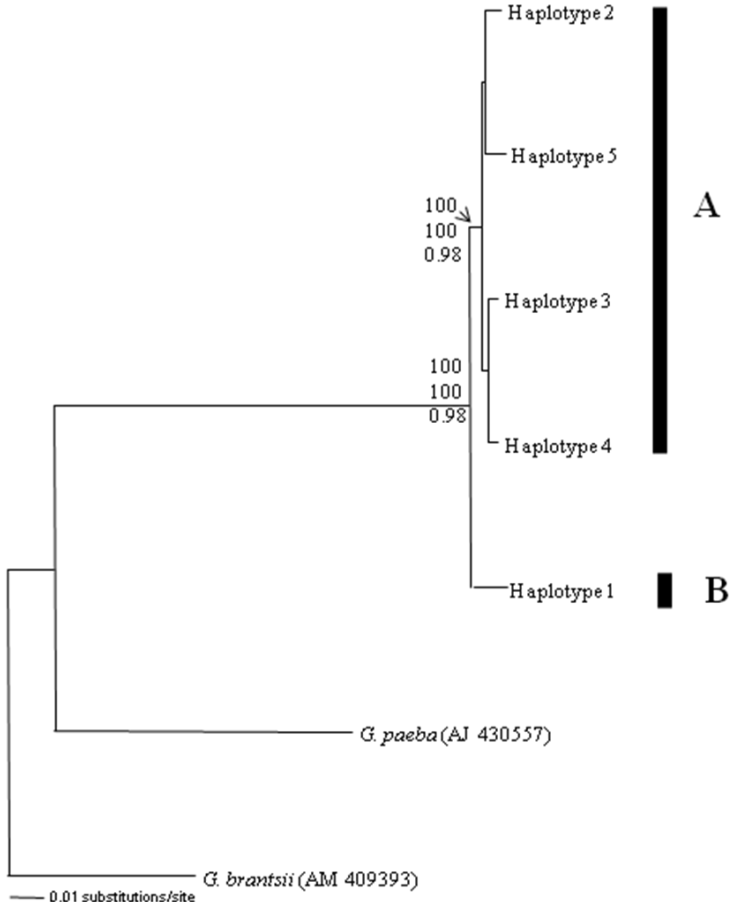

Figure 3.5: The maximum parsimony phylogram of *D. auricularis* using 394 bp of mtDNA cyt *b*. Above the nodes are the bootstrap values for NJ (top) and MP (bottom) and below the nodes are the posterior probabilities for the Bayesian Inference trees.

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Table 3.3: Uncorrected p-distance matrix indicating the divergence values (%) separating the five haplotypes of *D. auricularis* as well as outgroups. Distances are based on 394bp of the cytochrome *b* gene.

Haplotype number	**1**	**2**	**3**	**4**	**5**	***G. brantsii***	***G. paeba***
1							
2	1.522						
3	1.777	0.761					
4	1.777	0.761	0.507				
5	1.777	0.761	1.015	1.015			
G. brantsii	18.782	18.782	18.528	18.528	19.543		
G. paeba	20.812	20.558	20.558	20.558	20.558	14.721	

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3.3.3. Population structure of *G. paeba*

Twenty-six *G. paeba* specimens representing 12 localities were successfully amplified and sequenced (Table 3.1, Fig. 3.1B). The fragment contained 216 bp of partial mtDNA cyt *b* gene with base frequencies typical of the cyt *b* gene: A = 33.13 %, C =20.79 %, G = 13.92 % and T = 32.15 %. Of these sequences and excluding the outgroups, 207 characters were constant and nine were parsimony informative.

Eleven haplotypes were identified for the 26 specimens (see Fig. 3.6 and 3.7). Haplotype diversity is low (h = 0.4909 ± 0.1754) and nucleotide diversity (π) is 0.004714 ± 0.003846. A weak and non-significant positive correlation was found between geographic distance and F_{ST} values, indicating the absence of isolation by distance (r = 0.129; p = 0.16; Fig 3.8).

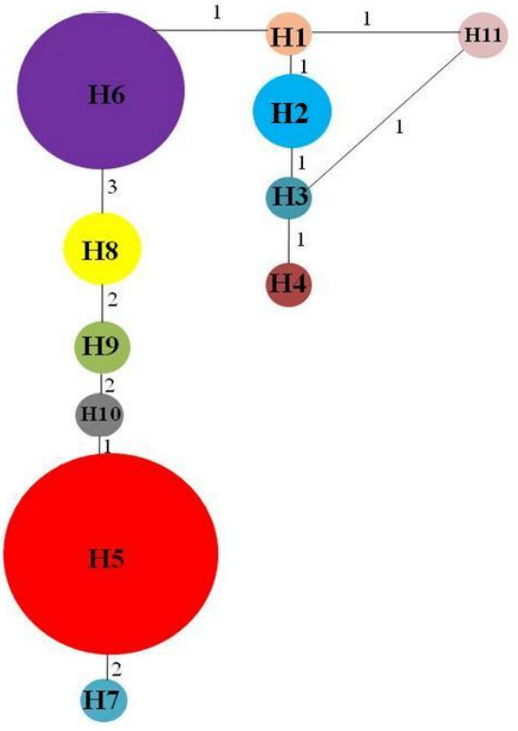

Figure 3.6: The haplotype network of *G.paeba* derived from 216bp of mtDNA cyt *b* of 26 specimens representing 12 localities. H1-H11 are the eleven haplotypes (connected at 95 % confidence limits) and the size of the circle is proportional to the number of individuals sharing that haplotype. The numbers of mutational steps separating haplotypes are indicated as numerical values

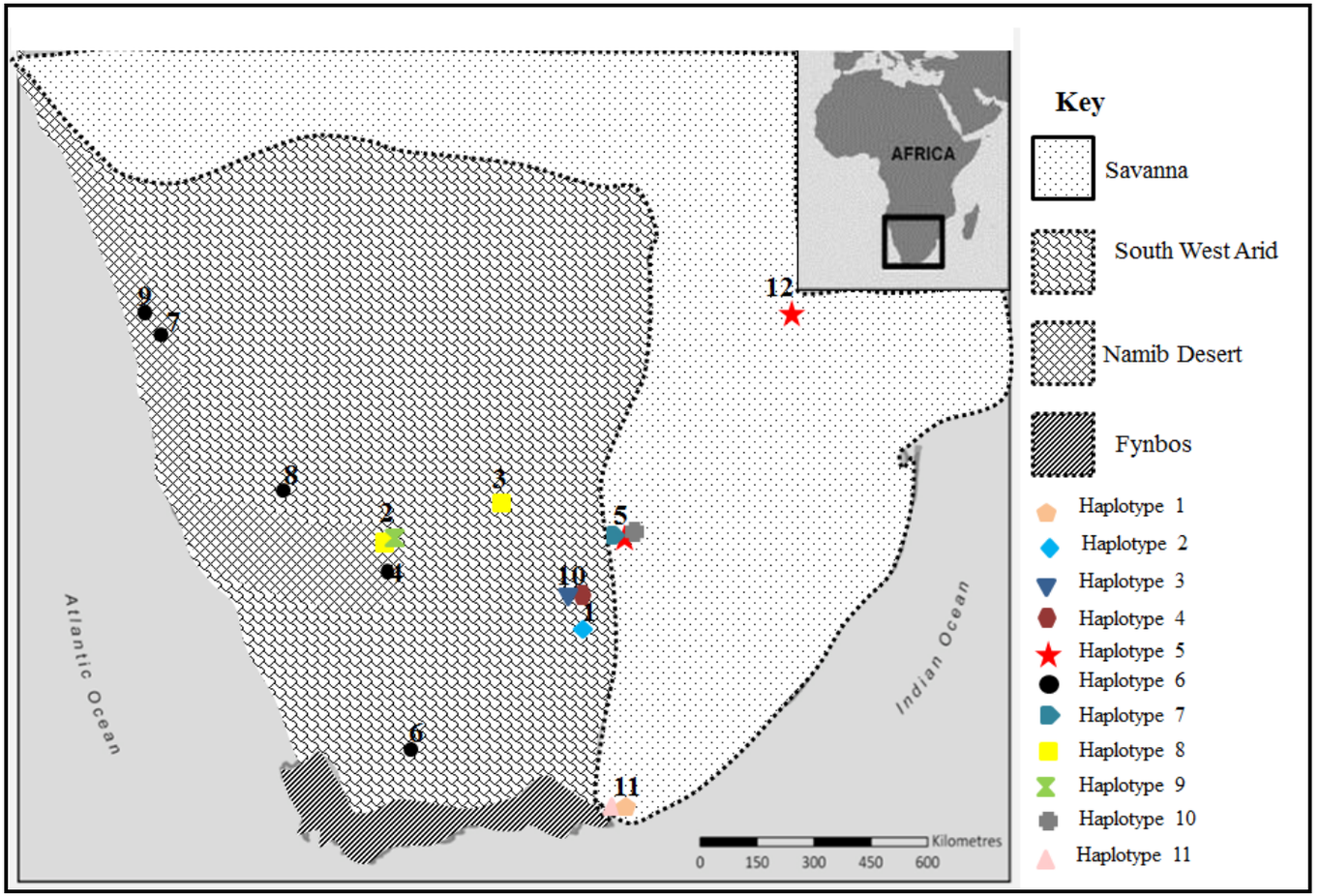

Figure 3.7: Geographic distribution ofhaplotypes of *G. paeba* derived from 216bp of mtDNA cyt *b* of 26 specimens representing 12 localities in the major biotic zones of southern Africa. Colours of haplotypes are proportional to the colour of haplotype in figure 3.6 and are represented by different shapes. Numbers represent localities and correspond with locality numbers in table 3.2.

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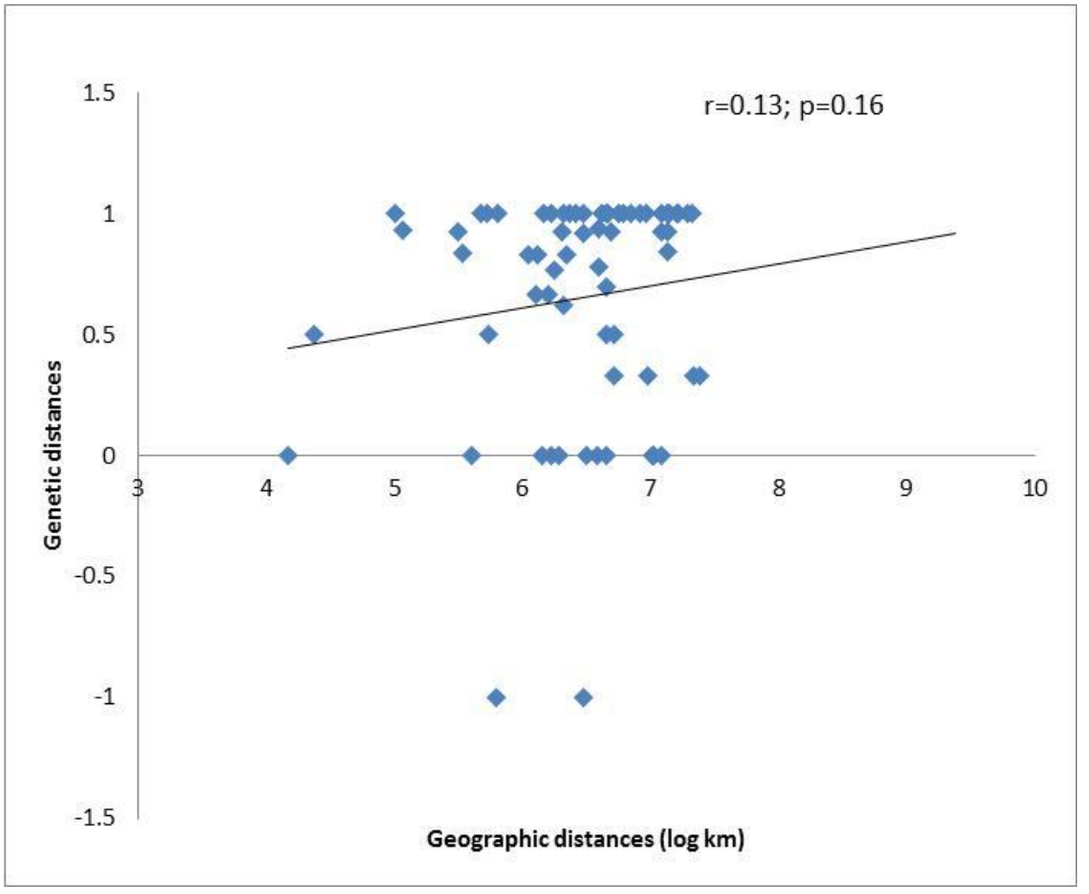

Figure 3.8: Scatter plot of geographic distances (log transformed km) plotted against genetic distances. Significance was determined through 1000 replicates.

3.3.4. Phylogenetic analysis of *G. paeba*

MODELTEST (jModeltest 0.1.1) selected HKY + I as the optimal model of evolution. The proportion of invariable sites (I) was 0.69. Maximum parsimony, neighbour joining and Bayesian inference retrieved nearly identical tree topologies containing two clades (Fig. 3.9). The sequence divergence between these clades ranges between 0.463 – 4.17% (Table 3.4). There was high statistical support for the ingroup (100% bootstrap and posterior probability of 1.00). The first clade (clade A) is comprised of six haplotypes from the Free State Province, three from Northern Cape Province and one from Limpopo Province (The second clade (clade B) is comprised of five haplotypes from Namibia, Free State Province, Northern Cape Province and Eastern Cape Province. However, the clades are not grouped according to the biotic zones of southern Africa.

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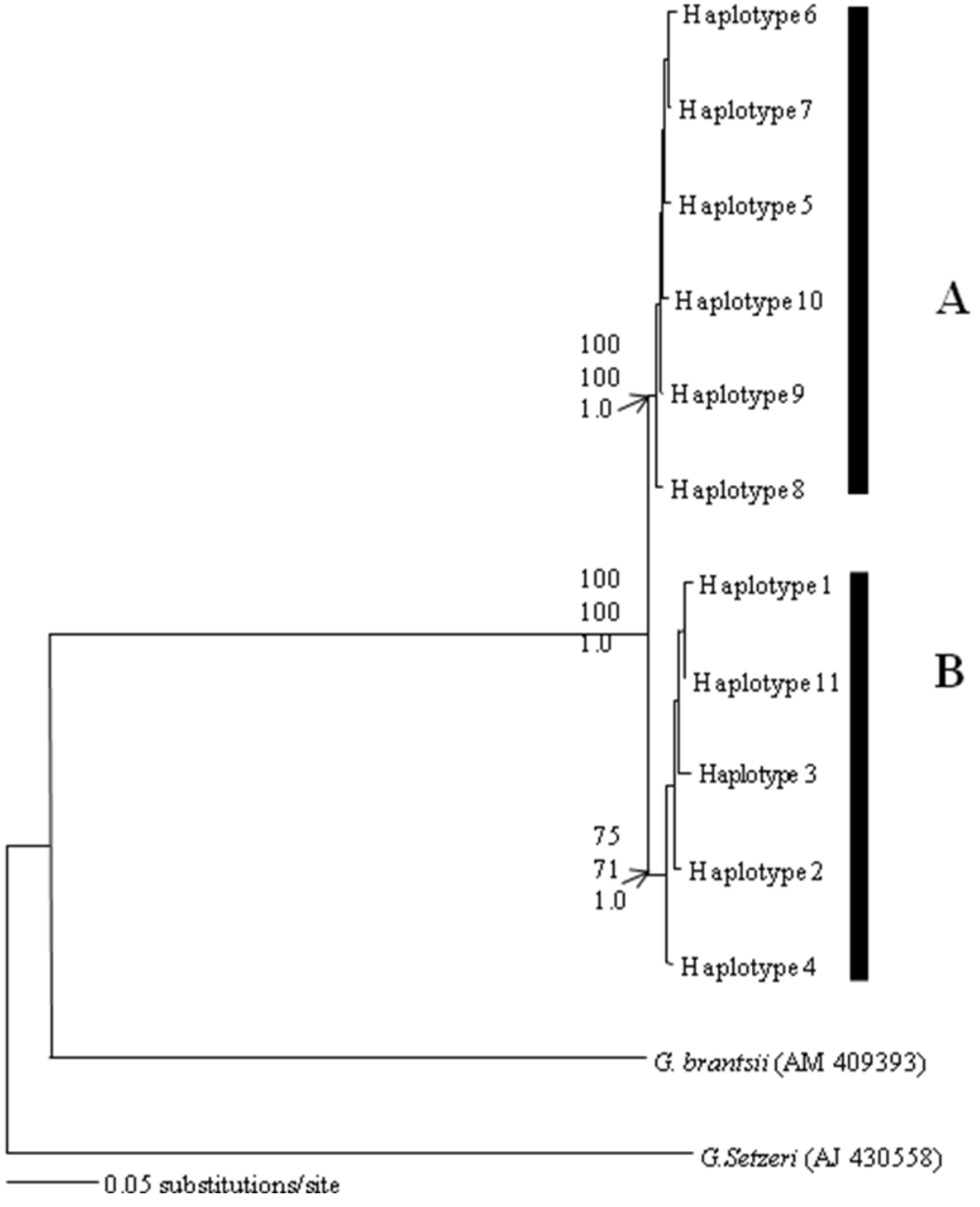

Figure 3.9: The maximum parsimony phylogram of *G. paeba* using 216bp of mtDNA cyt *b*. Above the nodes are the bootstrap values for NJ (top) and MP (bottom) and below the nodes are the posterior probabilities from the Bayesian Inference topologies

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Table 3.4: Uncorrected ("p") distance matrix indicating divergence values (%) of *G. paeba* using 216 bp of cyt *b*. Outgroups were included for comparative purposes

Haplotype number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	***G. setzeri***	***G. brantsii***
1	-												
2	1.39	-											
3	0.93	1.39	-										
4	2.31	0.93	1.39	-									
5	3.70	3.24	3.70	2.31	-								
6	2.78	3.24	3.70	3.24	0.93	-							
7	3.24	2.78	4.17	2.78	0.46	0.46	-						
8	3.70	2.31	2.78	1.39	0.93	1.85	1.39	-					
9	3.72	2.79	2.79	1.86	0.47	0.93	0.93	0.47	-				
10	3.70	2.31	3.70	2.31	0.93	0.93	0.46	0.93	0.47	-			
11	0.46	0.93	1.39	1.85	3.24	2.31	2.78	3.24	3.26	3.24	-		
G. setzeri	72.69	73.15	73.61	73.15	72.69	71.76	72.22	73.15	73.02	72.69	72.22	-	
G. brantsii	65.74	66.20	65.74	65.74	65.28	64.81	65.28	64.81	65.12	65.28	65.74	71.30	-

3.4 Discussion

3.4.1 Main findings

Desmodillus auricularis haplotype network consists of five haplotypes and phylogenetic analysis retrieved two monophyletic clades: clade A comprising four haplotypes and clade B consists of only one haplotype within their entire distribution. The sequence divergence between these two clades ranged between 1.52 and 1.77%, which indicates that *D. auricularis* is a monotypic species compared to the outgroup taxa which differ by 18.78% and 20.81% sequence divergence. *Gerbillurus paeba* also contains two clades. Clade A is comprised of six haplotypes (13 individuals) from Free State Province, three from Northern Cape Province and one from Limpopo province. Clade B is comprised of five haplotypes representing Namibia, Free State Province, Northern Cape Province and Eastern Cape Province. The sequence divergence between these clades ranges between 0.463 – 4.17% which were lower than the threshold for species delimitation in gerbils (see below). However, the elevated intraspecific sequence data need to be interpreted with caution as they are based on short stretches of mtDNA sequence and low numbers of specimens.

3.4.2 Geographic structure of *D. auricularis* vs. *G. paeba*

Desmodillus auricularis and *G. paeba* are distributed sympatrically within the arid regions of southern Africa. Therefore, it is reasonable to assume that both species will have identical phylogeographic structures. Interestingly, although the Orange River constitutes a barrier to gene flow in mostly rocky dwelling species, as in *M. namaquensis* (Russo *et al*., 2010), it does not appear to have the same effect on *D. auricularis* and *G. paeba*. This may be due to insufficient sampling on either side of the river or possibly reflecting intermittent flow of the Orange River (with dry periods allowing movement of animals) which may have allowed gene flow across this apparent barrier.

Similar to the findings of Meyer *et al*. (2009), neither of the species included in this study displayed isolation by distance. The study by Meyer and co-workers were based on microsatellite markers and included populations from the south-western Kalahari Desert in South Africa. Their study suggested that dispersal in *G. paeba* is female biased and that dispersal has little influence

on the genetic structure of the species but that genetic diversity is predominantly caused by genetic drift. It is known that genetic drift becomes increasingly important in smaller populations (Nei,1987). The absence of isolation by distance is surprising as smaller mammals that inhabit continuous habitat are often characterized by isolation by distance pattern (e.g. *Macroscelidesproboscideusproboscideus*;Smit *et al*., 2010). As the sample sizes are very small, it is possible that this pattern is an artefact of sampling. In support of this, there is a positive (albeit weak) relationship between genetic distance and geographic distance, as would typically be expected from isolation by distance.

The different genetic patterns between the study species may be a reflection of the physiological adaptation. While the water conserving abilities for these two species were adequate for existence in the arid regions, *D. auricularis*is better adapted than *G. paeba* (Smithers, 1971; Louw, 1972; Christian, 1979b; Perrin and Curtis, 1980). This adaptation allows *D. auricularis* to travel further when foraging which could result in extensive gene flow over great distances (Nel, 1967; Christian, 1977; Christian, 1979a and b). Further, *D. auricularis* reproduces aseasonally (Graaff, 1981; Perrin *et al.*, 1999) which is in contrast to *G paeba* which reproduces seasonally and have a shorter life span than *D. auricularis* (Christian, 1979b). Other life history differences include reproductive output: although both have similar number of offspring per litter, the aseasonal breeding strategy of *D. auricularis* may result in more offspring. Further, *G. paeba* is generally a solitary species compared to the communal *D. auricularis*.

Altogether, these factors suggests that gene flow will be higher in *D. auricularis* as reflected in the genetic data of this study which retrieved shallow genetic distances between specimens from distant localities. Conversely, the physiological restrictions and dispersal limitations in *G. paeba* are conducive for less contact between demes resulting in relatively elevated sequence divergence values.

3.4.3 Subspecies delimitation of *D. auricularis*

The sequence divergence between sister species of the same genus *Gerbilliscus* ranges between 9.6 and 25.7% using cyt *b* (Colangelo *et al.*, 2007). Values separating clades range between 1.53 -1.77% which is far below the interspecific sequence divergence in gerbils. The low sequence divergence also falls below the threshold observed in other small mammals of 1.87% (Bradley

and Baker, 2001) and 1-40% between several genera of small mammals (John and Avise, 1998). Therefore, this data confirms the existence of one species of *D. auricularis* throughout its distribution.

3.4.4 Subspecies delimitation of *G. paeba*

According to Colangelo *et al (*2007), the sequence divergence between sister species of the same genus in gerbils (genus *Gerbilliscus*) ranges between 9.6 and 25.7% using 1140 bp of cyt *b* and, according to John and Avise (1998) it ranges between 1-40% between different genera of small mammals. The sequence divergence of *G. paeba* based on 216 bp in this study ranges from 0.463 - 4.17% which is much lower than values obtained for congeners (Table 3.6) for the same stretch of mtDNA. This clearly suggests existence of one species which is consistent with Wilson and Reeder (2005) who do not recognize any subspecies of *G. paeba*.

3.5 Conclusions

Since the two species occur sympatrically, it was hypothesized that they would be exposed to the same selection pressure and possibly have identical genetic signatures. The mtDNA data different fairly similar genetic structures and the difference in the number of haplotypes may be due to a combination of divergent social structures, physiological adaptations and habitat preference. Further, the sequence data suggest high gene flow between the populations of *D. auricularis* as suggested by the wide distribution of the common haplotype. With regards to *G. paeba*, the data do not support the recognition of four subspecies; this is clearly indicated by lower sequence divergence. However, this does not exclude the existence of the two lineages in *G. paeba*, which may be evident by increasing the sample sizes throughout their range.

Chapter 4
General Conclusions

This is the first study on two sympatric gerbil species, *D. auricularis* and *G.paeba* within southern Africa using both cranial morphometrics and molecular data. The use of both molecular and morphometric analyses to investigate potential geographic variation breaks in *D. auricularis* and *G.paeba* proved to be useful, even though interpretations were limited by low sample sizes.

Multivariate analysis confirms the absence of sexual dimorphism on both *D. auricularis* and *G. paeba*, which is common in gerbils (e.g. *G. setzeri* and *G. leucogaster*; De Graaff, 1981). Further, there is no cranial variation within *D. auricularis*, even though *G. paeba* showed slight differentiation. Differences between cranial morphology of these two sympatrically species may be attributed to habitat requirements, physiological adaptations and social structures. Multivariate analysis indicates that *D. auricularis* is a monotypic species and biotic zones of southern Africa do not represent more than one lineage within the species. Morphometrics analyses indicate that four subspecies of *G. paeba* that have been documented can be reduced to two subspecies: *G. p. paeba* which has a widest distribution and consist of specimens from the South West Arid zone and*G. p. coombsi* comprise specimens from the Southern Savanna Woodlands (Soutpansberg area). Diagnostic variables distinguishing *G. paeba* subspecies are rostral height and mandibular length.

The results from mtDNA cyt *b* gene sequence data clearly indicate that despite a wide distribution, *D.auricularis* is monotypic. Further, even though two clades were retrieved, there were relatively low sequence divergences among the clades. Clade A is comprised of four haplotypes and clade B which is ancestral haplotype is consisted of a predominantly common haplotype which has the widest distribution. However, the phylogeographic patterns observed in *G. paeba* were not comparable to those of *D. auricularis*; even though two clades were similarly retrieved (the clades do not have the identical distribution). Sequence divergences separating the two clades are lower than the intrapopulation and intrasubspecific ranges of rodents and lower

than the sister species of gerbils (Bradley and Baker, 2001, Colangelo *et al.*, 2007). Therefore sequence divergence data or clades only suggest limited geographic variation in *G. paeba*.

Broadly, the morphometrics and molecular data are partly congruent. Both indicate single lineage (certain for *D. auricularis*) and perhaps two (sequence data) or one (morphological) lineage(s) for *G. paeba*. Overall, the data does not support the null hypothesis suggesting division along biotic zones or geographical barriers within southern Africa. Most certainly, the biotic zones that comprise the regions of southern Africa do not appear to have any effect on both gene flow and morphological structure. This has also been observed in plain dwelling species of the arid regions (Smit *et al*., 2010; Edwards *et al*., 2011). In contrast, habitat preferences were probably responsible for shaping the variation observed in these arid dwelling species.

Finally, the approach taken in this study may have played a role in the outcome of this study. For instance, the relative lack of variation detected using morphometric data may mask cranial differences that can only be observed using the more powerful geometric morphometrics analysis. Similarly, shallow sequence divergences might reflect the conservatism inherent in the cyt *b* data (including short DNA sequences), and therefore not a true reflection of lineages that may be uncovered using more variable markers such as the control region and microsatellite loci. In a nut shell geometric morphometrics and faster evolving genetic markers may provide better resolution than linear morphometrics and the conserved cyt *b* which is particularly useful for phylogenetic inferences.

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Appendices

Appendix 1: List of specimens used in morphometrics study with specimen number, museum identity numbers, locality, country, GPS coordinates, collection date, sex of *D. auricularis* (A) and *G. paeba* (B).

A

Specimen number	Museum id number	Locality	Country	Coordinates	Collection Date	Gender
1	TM 63	Kuruman	South Africa	27° 27′ 00″ S 23° 25′ 59" E	01-Sep-03	M
2	TM 23026	47.2 Kuruman-Dikgatlong	South Africa	27° 07′ 30″S 23° 07′ 30" E	29-Aug-47	M
3	TM 22981	Dikgatlong-Deben Rd	South Africa	27° 52′ 30″ S 22° 52′ 30" E	17-Aug-50	F
4	TM 22986	3 MI Vryburg-Kuruman	South Africa	26° 57′ 00″ S 24° 43′ 59" E	22-Sep-47	M
5	TM 22975	Paddou	South Africa	25° 37′ 30″ S 23° 52′ 30" E	08-Sep-57	F
6	TM 22968	Esperance Farm	South Africa	25° 37′ 30″ S 23° 52′ 30" E	01-Jan-63	M
7	TM 32712	35KM E,Farm Avondale	South Africa	28° 22' 00" S 21° 35' 00" E	03-Nov-80	M
8	TM 33734	NW, Farm Cnydas 12KM	South Africa	28° 19' 00" S 20° 34' 00" E	27-Oct-84	M
9	TM 32464	NW, Farm Cnydas 12KM	South Africa	28° 19' 00" S 20° 34' 00" E	07-Oct-80	M
10	TM 23036	Olifantshoek-Khosis, 18.5	South Africa	27° 19′ 35″ S 22° 04′ 17" E	03-Oct-47	F
11	TM 23062	Olifantshoek Commonage	South Africa	27° 19′ 35″ S 22° 04′ 17" E	26-Sep-56	
12	TM 23043	Deben-Vuilnek 11.8	South Africa	27° 19′ 35″ S 22° 04′ 17" E	20-Oct-47	M
13	TM 23013	Twee Rivieren	South Africa	26° 22′ 30″ S 20° 37′ 30" E	19-Nov-62	F
14	TM 23008	Twee Rivieren	South Africa	26° 22′ 30″ S 20° 37′ 30" E	20-Nov-62	M
15	TM 22973	Twee Rivieren	South Africa	26° 22′ 30″ S 20° 37′ 30" E	22-May-56	F
16	TM 22991	Twee Rivieren	South Africa	26° 22′ 30″ S 20° 37′ 30" E	19-Nov-62	M
17	TM 22970	Twee Rivieren	South Africa	26° 22′ 30″ S 20° 37′ 30" E	10-Jun-63	F
18	TM 22984	Griquatown 11.9	South Africa	28° 52′ 30″ S 22° 22′ 30" E	05-Oct-47	F
19	TM 23054	Griquatown 11.9	South Africa	28° 52′ 30″ S 22° 22′ 30" E	05-Oct-47	F
20	TM 23006	Griqautown 11.9	South Africa	28° 52′ 30″ S 22° 22′ 30" E	05-Oct-47	M
21	TM 22999	8 MI N Vaal Bridge,Windsorton	South Africa	26° 57′ 00″ S 24° 43′ 59" E	24-Sep-47	M
22	TM 12549	Panfontein 270	South Africa	27° 35' 00" S 25° 27' 00" E	13-Aug-53	M
23	TM 6902	Rietfontein	South Africa	26° 22' 30" S 20° 37' 30" E	03-Jul-32	M
24	TM 5387	Bloemhof	South Africa	27° 39' 00" S 25° 31' 00" E	19-Jun-28	M
25	TM 5389	Vryburg	South Africa	26° 57' 00" S 24° 43' 59" E	19-Jun-28	M
26	TM 27240	20km SE Prieska,	South Africa	29° 40′ 00″ S 22° 45′ 00" E	05-Feb-	F

		Farm Keikamspoort 71				77	
27	TM 4197	Prieska	South Africa	29° 40' 00" S	22° 45' 00" E		M
28	TM 32458	25km SW Kenhardt, Farm Vissersvil	South Africa	23° 07' 30" S	15° 37' 30" E	05-Oct-80	M
29	TM 23001	Douglas-Hopetown, 28.4	South Africa	29° 44′ 52″ S	24° 18′ 59" E	06-Oct-47	F
30	TM 23038	4.6 Douglas-Hopetown	South Africa	29° 44′ 52″ S	23° 18′ 59" E	05-Oct-47	M
31	TM 39356	Tanqua Karoo Nat. Park, Folmoesfontein, Farm 1097	South Africa	32° 17' 00" S	19° 52' 00" E	26-Sep-87	F
32	TM 39358	Tanqua Karoo Nat. Park, Folmoesfontein, Farm 1097	South Africa	32° 17' 00" S	19° 52' 00" E	26-Sep-87	M
33	TM 39365	Tanqua Karoo Nat. Park, Folmoesfontein, Farm 1097	South Africa	32° 17' 00" S	19° 52' 00" E	26-Sep-87	F
34	TM 39366	Tanqua Karoo Nat. Park, Folmoesfontein, Farm 1097	South Africa	32° 17' 00" S	19° 52' 00" E	26-Sep-87	F
35	TM 33769	40km E,FarmKangnas	South Africa	29° 34' 00" S	18° 21' 00" E	13-Oct-81	F
36	TM 28121	Farm Kangnas	South Africa	29° 35' 00" S	18° 21' 00" E	15-Oct-77	M
37	TM 28145	Farm Kangnas	South Africa	29° 34' 00" S	18° 21' 00" E	17-Oct-77	F
38	TM 28157	Farm Kangnas	South Africa	29° 34' 00" S	18° 21' 00" E	17-Oct-77	M
39	TM 28104	10km NNE Bitterfontein, Farm Kersbos	South Africa	30° 57' 00" S	18° 12' 00" E	13-Oct-77	F
40	TM 8086	Klipfontein	South Africa	29° 52′ 30″ S	17° 52′ 30" E	19-Aug-37	M
41	TM 8088	Ookiep	South Africa	29° 37′ 30″ S	17° 52′ 30" E	24-Aug-37	F
42	TM 8796	Vredendal	South Africa	31° 37′ 30″ S	18° 37′ 30" E		M
43	TM 5073	Atties	South Africa	31° 37′ 30″ S	18° 22′ 30" E	17-Sep-27	M
44	TM 5082	Klaver	South Africa	31° 46′ 59″ S	18° 37′ 00" E	06-Nov-26	F
45	TM 4744	Vlermuisklip	South Africa	31° 33′ 20″S	18° 20′ 28″ E	01-Sep-26	F
46	TM 23019	Amanzi	South Africa	33° 37′ 30″ S	25° 37′ 30" E	20-Oct-59	M
47	TM 23065	Amanzi	South Africa	33° 37′ 30″ S	25° 37′ 30" E	20-Oct-59	F
48	TM 23035	3.6 Fort Beaufort-Fort Brown	South Africa	32° 37′ 17″ S	26° 37′ 53" E	26-Jun-49	M
49	TM 8797	Eendekuil	South Africa	32° 41′ 17″ S	18° 53′ 02" E	23-Mar-37	M
50	TM 8798	Eendekuil	South Africa	32° 41′ 17″ S	18° 53′ 02" E	24-Mar-37	F
51	TM 23004	Havenga Bridge, Fauresmith	South Africa	29° 44′ 52″ S	24° 18′ 59" E	30-Jul-62	M
52	TM 23018	3.2 MI Sandflats on Rd to Ceverney	South Africa			10-Feb-60	M
53	TM 23024	3.6 Fort Beaufort-Fort Brown	South Africa	32° 37′ 17″ S	26° 37′ 53" E	1949/06/61	F
54	TM 22980	Kariega Station	South Africa	33° 23′ 24″ S	25° 26′ 22" E	24-Aug-63	M
55	TM 4329	Swakopmund	Namibia	22° 40′ 22″S	14° 31′ 39″ E	19-Feb-25	F
56	TM 4330	Swakopmund	Namibia	22° 40′ 22″S	14° 31′ 39″ E	20-Feb-25	M

57	TM 4300	Quickborn	Namibia	21° 07′ 30″ S 17° 07′ 30" E	02-Oct-24	M
58	TM 5698	Gababis	Namibia	22° 22' 30" S 18° 52' 30" E	19-Dec-28	M
59	TM 7614	Ondonga	Namibia	17° 37' 30" S 16° 07' 30" E	16-Feb-33	M
60	TM 7616	Ondonga	Namibia	17° 37' 30" S 16° 07' 30" E		M
61	TM 7624	Ongandjera	Namibia	18° 07′ 30″ S 14° 37′ 30" E		M
62	TM 8083	Barbi	Namibia	18° 07′ 30″ S 14° 37′ 30" E	25-Jul-37	M
63	TM 14794	Gababeb	Namibia	23° 37' 30" S 15° 07' 30" E	21-May-65	F
64	TM 15137	De Waal	Namibia	32° 37' 30" S 18° 52' 30" E	15-Oct-65	F
65	TM 15138	Palmenhorst, Swakop River	Namibia	22° 41′ 37″ S 14° 54′ 00" E	26-Oct-65	M
66	TM 15136	Unknown Locality	Namibia	18° 22′ 30″ S 12° 22′ 30" E	06-Oct-65	F
67	TM 22993	Keetmanshoop Golf Course	Namibia	26° 34′ 20″ S 18° 08′ 02" E	11-Mar-50	F
68	TM 22972	4 MI Nwaus	Namibia	26° 22′ 30″ S 16° 37′ 30" E	12-Jan-58	F
69	TM 22989	4 MI Nwaus	Namibia	26° 22′ 30″ S 16° 37′ 30" E	12-Jul-58	F
70	TM 23025	Zesfotein	Namibia	19° 07′ 30″ S 13° 37′ 30"E	20-Jul-51	M
71	TM 23057	Farm 415	Namibia	21° 52′ 30″ S 19° 07′ 30" E	23-Feb-50	M
72	TM 28869	Ganab,Namib Park	Namibia	23° 07' 30" S 15° 37' 30" E	27-May-78	F
73	TM 28874	Namib Park, Ganab	Namibia	23° 07' 30" S 15° 37' 30" E	28-May-78	M
74	TM 28897	Namib Park, Ganab	Namibia	23° 07' 30" S 15° 37' 30" E	31-May-78	
75	TM 32688	Huns 106	Namibia	27° 23' 00" S 17° 23' 00" E	31-Oct-80	F
76	TM 37519	Gellap-Ost No. 3, 16km N	Namibia	22° 26' 00" S 18° 06' 00" E	27-Oct-84	F
77	TM 37520	Gellap-Ost No. 3, 16km N	Namibia	22° 26' 00" S 18° 06' 00" E	27-Oct-84	M
78	TM 38811	Klein Aus, 1km W	Namibia	26° 39' 00" S 16° 10' 00" E	Nov-85	F
79	TM 38820	Klein Aus, 1km W	Namibia	26° 39' 00" S 16° 10' 00" E	Nov-85	M
80	TM 12544	Panfontein 270	Botswana	27° 35' 00" S 25° 27' 00" E	13-Aug-53	F
81	TM 6370	Motlhatlogo	Botswana	20° 22' 30" S 22° 52' 30" E		
82	TM 6371	Motlhatlogo	Botswana	20° 22' 30" S 22° 52' 30" E		
83	TM 7013		Botswana			
84	TM 23048	Sehithwa B.P	Botswana	20° 22′ 30″ S 22° 37′ 30" E	12-Nov-58	F
85	TM 22976	Sebitwa Lake Ngani	Botswana	20° 22′ 30″ S 22° 52′ 30" E	13-Dec-44	F
86	TM 22987	Makalamabeai	Botswana	20° 22′ 30″ S 23° 52′ 30" E	09-Jul-65	F
87	TM 23046		Botswana			

B

Specimen number	Museum id numbers	Locality names	Country	Coordinates	Collection Date	Gender
1	TM 4810	Olifants and Doorn Rivers	South Africa	31° 37' 30" S 18° 22' 30" E		F
2	TM 4812	Olifants and Doorn Rivers	South Africa	31° 37' 30" S 18° 22' 30" E		M
3	TM 29506	Karoo National Park	South Africa	32° 20' 40" S 22° 33' 00"E	21-Jan-79	M
4	TM 29602	Karoo National Park	South Africa	32° 20' 40" S 22° 33' 00"E	30-Jan-79	F
5	TM 6895	Upington, 76mi N	South Africa	27° 52′ 30″ S 20° 22′ 30" E	02-Jul-32	M
6	TM 16864	Nossob camp, Gordornia	South Africa	26° 22′ 30″ S 20° 37′ 30" E	20-Jan-70	F
7	TM 16882	Nossob camp, Gordornia	South Africa	26° 22′ 30″ S 20° 37′ 30" E	19-Jan-70	F
8	TM 27218	Farm Keikamspoort 71	South Africa	29° 50' 00" S 22° 47' 00" E	04-Feb-77	M
9	TM 27429	Augrabies Falls National Park	South Africa	28° 37' 30" S 20° 22' 30" E	06-May-77	M
10	TM 27444	Augrabies Falls National Park	South Africa	28° 37' 30" S 20° 22' 30" E	07-May-77	F
11	TM 37363	Farm Sudbury	South Africa	22° 52' 30" S 29° 22' 30" E	05-Jun-69	F
12	TM 37464	Langjan Nat Res, 20KM N, Soutpansberg	South Africa	22° 53' 53" S 29° 36' 59" E	27-Sep-84	M
13	TM 5380	Montrose Estates	South Africa	22° 54' 00" S 29° 37' 00" E	07-Jul-28	F
14	TM 39325	Farm 153	South Africa	29° 13' 00" S 17° 04' 00" E	13-Sep-87	F
15	TM 39326	Farm 153	South Africa	29° 13' 00" S 17° 04' 00" E	13-Sep-87	M
16	TM 43629	Sendlingsdrift, 14km E	South Africa	28° 07' 36" S 16° 59' 13" E	30-Sep-92	M
17	TM 43633	Sendlingsdrift, 14km E	South Africa	28° 07' 36" S 16° 59' 13" E	30-Sep-92	F
18	TM 43639	Sendlingsdrift, 14km E	South Africa	28° 07' 36" S 16° 59' 13" E	29-Sep-92	F
19	TM 45735	Molopo National Reserve	South Africa	25° 53' 00" S 22° 54' 35" E	21-Aug-97	F
20	TM 37408	Dikgatlong-Deben Rd, 25.7miI	South Africa	27° 27' 00" S 22° 25' 59" E	17-Aug-50	M
21	TM 33774	Farm Kangnas, 40km E	South Africa	29° 34' 00" S 18° 21' 00" E	13-Nov-81	M
22	TM 33784	Farm Kangnas, 40km E	South Africa	29° 34' 00" S 18° 21' 00" E	14-Nov-81	F
23	TM 34056	Farm Kleinfontein	South Africa	31° 07' 00" S 25° 12' 00" E	02-Dec-81	F
24	TM 34120	Farm Kleinfontein	South Africa	31° 07' 00" S 25° 12' 00" E	04-Dec-81	F
25	TM 37416	Sunday River Mouth	South Africa	33° 37' 30" S 25° 52' 30" E		
26	TM 37331	Sundays River Mouth, E side	South Africa	25° 52' 30" S 33° 37' 30" E	18-Oct-59	M
27	TM 37332	Sundays River Mouth	South Africa	25° 52' 30" S 33° 37' 30" E	18-Oct-59	M
28	TM 37333	Sundays River Mouth	South Africa	25° 52' 30" S 33° 37' 30" E	18-Oct-59	F
29	TM 37329	Sundays River Mouth, E side	South Africa	25° 52' 30" S 33° 37' 30" E	18-Oct-59	M
30	TM 37330	St. George Strand	South Africa	25° 52' 30" S 33° 37' 30" E	15-Mar-59	F
31	TM 37339	Vredendal	South Africa	31° 37' 30" S 18° 37' 30" E	29-Sep-30	F
32	TM 28066	Farm Kersbos	South Africa	30° 57' 00" S 18° 12' 00" E	11-Oct-77	F
33	TM 28070	Farm Kersbos	South Africa	30° 57' 00" S 18° 12' 00" E	11-Oct-77	F
34	TM 28079	Farm Kersbos	South Africa	30° 57' 00" S 18° 12' 00" E	12-Oct-77	F

35	TM 27604	GababebDeru	Namibia	23° 37' 30" S 15° 07' 30" E	01-Aug-77	M
36	TM 27399	Goraas, Farm FrQ 2.9	Namibia	31° 10' 00" S 21° 35' 00" E	14-Feb-77	M
37	TM 10986	Seeheim, Fish River	Namibia	26° 52′ 30″ S 17° 52′ 30" E	23-Jul-51	M
38	TM 28850	Unknown Locality	Namibia	23° 52′ 30″ S 15° 07′ 30" E	24-May-78	F
39	TM 32599	Farm Rheinveils, 35km SSW	Namibia	26° 57' 00" S 17° 56' 00" E	16-Oct-80	F
40	TM 32638	Oranjemund: Diamond Area	Namibia	28° 32' 58" S 16° 25' 32" E	26-Oct-80	F
41	Tm 32639	Oranjemund: Diamond Area	Namibia	28° 32' 58" S 16° 25' 32" E	27-Oct-80	M
42	TM 37377	Below Sar Bridge at Seeheim 1/4 MI	Namibia	26° 52′ 30″ S 17° 52′ 30" E	03-Mar-50	F
43	TM 37378	Luderitz	Namibia	23° 33′ 35″ S 15° 02′ 25" E	16-Mar-50	F
44	TM 37400	Katara	Namibia	17° 52′ 30″ S 18° 52′ 30" E	11-Aug-65	F
45	TM 38267	Cape Fria	Namibia	18° 25′ 39″ S 12° 14′ 43" E	18-Dec-84	M
46	TM 15273	Tierputs, Ghanzi	Botswana	21° 52′ 30″ S 21° 22′ 30" E	04-Nov-61	M
47	TM 16557	Okwa Valley	Botswana	22° 22′ 30″ S 23° 07′ 30" E	02-Jul-69	F
48	TM 16559	Okwa Valley	Botswana	22° 22′ 30″ S 23° 07′ 30" E	02-Jul-69	M

Appendix 2: Basic statistics [arithmetic mean, standard deviation (Std. Dev.), range and coefficient of variation (CV %) with Haldane's (1955) correction] and the results of test of normality [Kolmogorov-Smirnov (K-S), Skewness (g1) and Kurtosis (g2)] of *D. auricularis* (A) and *G. paeba* (B).

A

Variables	Mean	Std.Dev.	CV%	Range	g1	g2	K-S
GLS	1.536225	0.016881	1.098862	0.086367	-0.527035	0.18713	0.09014
BB	1.169166	0.013611	1.164163	0.052494	0.337753	-0.4974	0.0649
IB	0.761487	0.021886	2.874113	0.122858	0.52669	0.91272	0.05346
RW	0.669259	0.021547	3.219531	0.099217	-0.104997	-0.3649	0.0585
RH	0.778149	0.022477	2.888521	0.117453	-0.382507	0.42823	0.0873
RL	1.01912	0.023233	2.279712	0.112447	-0.196012	-0.045	0.06078
TBL	1.117853	0.02073	1.854448	0.117988	-0.359849	0.64529	0.07005
UTR	0.654652	0.020627	3.150834	0.082698	0.356538	-0.846	0.10548
BM[1]	0.212626	0.025292	11.89506	0.113943	-0.015997	-0.4764	0.07703
PW	0.49056	0.03474	7.081703	0.177306	-0.602605	0.61197	0.06414
OH	1.001992	0.020554	2.051314	0.099792	-0.830432	0.62973	0.10726
LMC	0.626556	0.020654	3.296433	0.113855	0.231164	0.20629	0.07193
HM	0.827406	0.01776	2.146467	0.104005	-0.822638	1.19903	0.12682
ML	1.262732	0.01982	1.569613	0.094158	-0.524274	-0.0458	0.08035

B

Variables	Mean	Std. Dev.	CV %	Range	g1	g2	K-S
GLS	1.463553	0.015951	1.089882	0.084591	-0.83996	1.672272	0.07111
BB	1.101846	0.013036	1.183105	0.0606	-0.536477	0.396797	0.09862
IB	0.754616	0.022118	2.931027	0.102029	0.383636	0.068334	0.07751
RW	0.584817	0.021685	3.707998	0.115688	0.265511	0.96444	0.0781
RH	0.705856	0.026168	3.707272	0.154263	-0.786098	1.407819	0.06216
RL	0.930123	0.027409	2.946815	0.140286	-0.703703	0.882456	0.097
TBL	0.928616	0.020728	2.232139	0.111625	-0.404464	0.905086	0.09355
UTR	0.620363	0.020701	3.336917	0.079931	0.461499	-0.61957	0.09385
BM[1]	0.176242	0.021743	12.33701	0.102837	-0.462325	0.097143	0.07657
PW	0.480387	0.026304	5.475585	0.124514	-0.442858	0.270915	0.11314
OH	0.922637	0.017127	1.85631	0.098861	0.305845	1.291918	0.04854
LMC	0.604681	0.023387	3.867659	0.109144	0.633713	0.976208	0.10959
HM	0.721331	0.029951	4.152185	0.155525	-0.721243	1.351581	0.10091
ML	1.149314	0.019171	1.668038	0.087119	-0.457708	-0.05459	0.07061

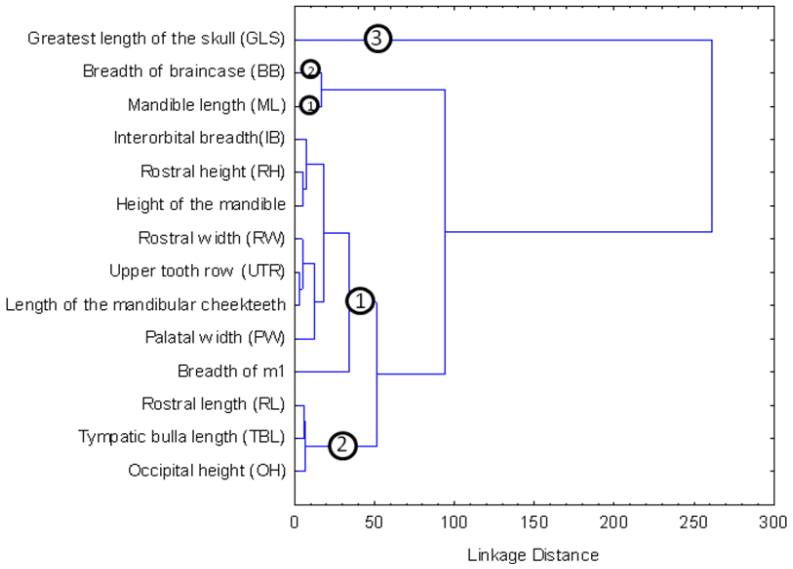
Appendix 3: Percent measurement error (% ME) and coefficient variation of 14 cranial measurements of 11 individuals for *G. paeba* (A) and *D. auricularis* (B). CV_{WI} = overall variation within-individuals error, CV_{BI} = overall variation between-individual error for each character.

A

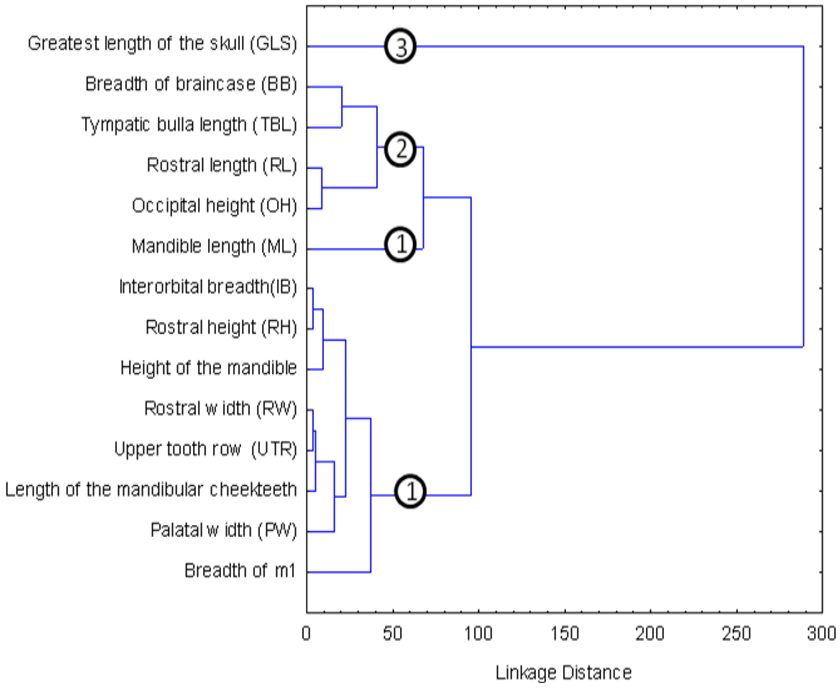
Variables	CV_{WI}	CV_{BI}	%ME
GLS	0.01	3.84	0.00158
BB	0.01	1.87	0.005141
IB	0.11	4.68	0.256594
RW	0.17	3.5	1.800238
RH	0.01	4.23	0.006282
RL	0.05	6.55	0.036045
TBL	0.00	2.54	0
UTR	0.05	3.5	0.09025
BM[1]	0.07	2.65	0.681199
PW	0.98	7.18	13.76455
OH	0.01	3.06	0.004371
LMC	0.35	4.31	7.035807
HM	0.53	5.15	10.89306
ML	0.01	4.93	0

B

Variables	CV_{BI}	CV_{WI}	%ME
GLS	4.94	0.09	0.24477
BB	1.92	0.41	36.2765
IB	4.65	0.43	6.83623
RW	5.32	0.42	1.26762
RH	4.24	0.5	1.07852
RL	4.93	0.15	0.61131
TBL	3.91	0.22	0.00774
UTR	4.03	1.12	11.1378
BM[1]	3.93	0.92	3.06781
PW	5.95	1.09	11.6884
OH	4.22	0.84	20.4363
LMC	4.03	0.51	15.7788
HM	5.7	0.94	24.4657
ML	5.84	0.16	0.50067



A



B

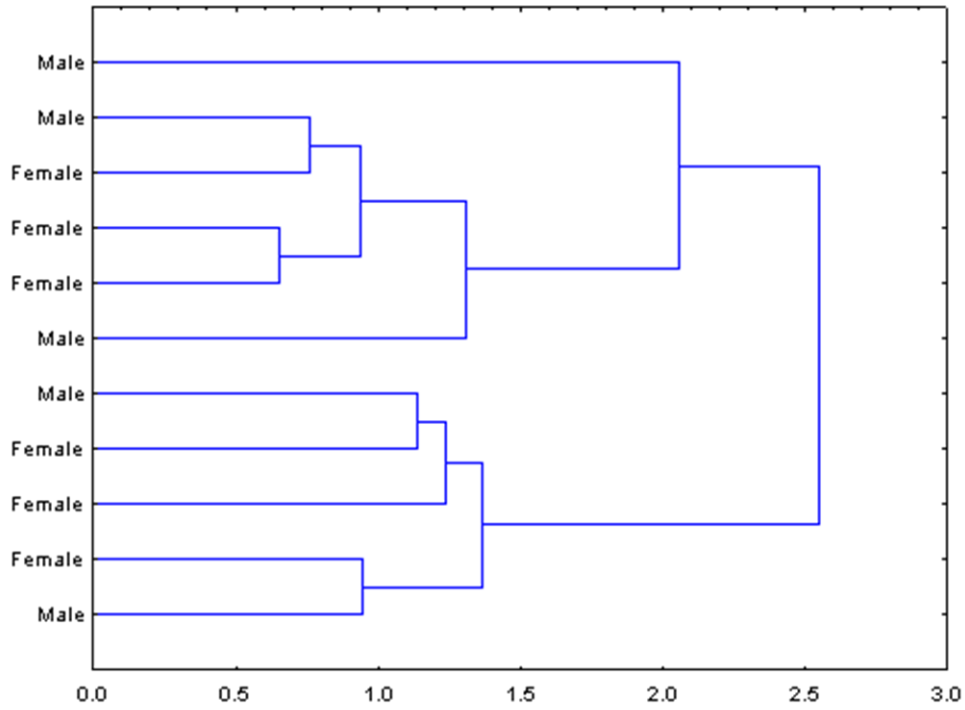
Appendix 4: Phenogram generated from hierarchical cluster analysis using an unweighted pair group mean analysis (UPGMA) clustering algorithm illustrating relationship of 14 cranial variables of *G. paeba* (A) and *D. auricularis* (B). Number represents functional sets as described in Chimimba and Dippenaar (1994)

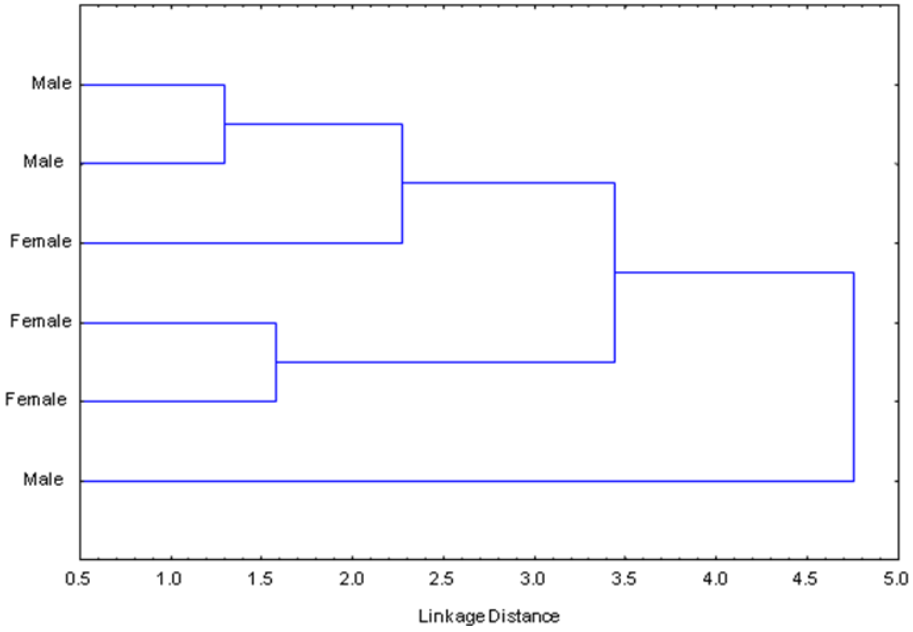
Appendix 5: Results of Levene's homogeneity and one-way ANOVA tests for sexual dimorphism (Sex) and tooth wear class variation (TW) in cranial measurements of *G. paeba* with mean size differences for significantly different measurements expressed as a percentage (%). df = degrees of freedom, P = significance and *indicate significance at P<0.05

Levene's		GLS	IB	RH	RW	RL	TBL	ML	BM[1]	BB	OH	UTR	LMC
1-way ANOVA	F	3.556205	0.138707	3.996212	1.7599	4.173852*	0.582583	1.235979	0.27702	0.050738	0.801062	3.087124	0.699763
	df	1	1	1	1	1	1	1	1	1	1	1	1
	P	0.066252	0.711443	0.052101	0.191805	0.047360*	0.449568	0.272571	0.601429	0.822876	0.375877	0.086203	0.407598
sex													
	F	0.645448	0.821621	0.042136	0.185112	0.82911	2.143341	0.053766	1.068695	0.34232	0.433656	0.514254	1.092128
	df	1	1	1	1	1	1	1	1	1	1	1	
	P	0.426268	0.369878	0.838353	0.669216	0.367726	0.150635	0.817762	0.307156	0.561624	0.513795	0.477273	0.301978
TW	Df	1	1	1	1	1	1	1	1	1	1	1	1
	F	0.13577	0.000316	0.064391	0.081587	0.647511	0.458744	0.399492	0.025612	0.008303	0.150049	1.54697	3.305318
	P	0.714331	0.985903	0.800896	0.776529	0.425431	0.501839	0.530698	0.873601	0.927819	0.7004	0.220321	0.076027

Appendix 6: Results of Levene's homogeneity and one-way ANOVA tests for sexual dimorphism (Sex) and tooth wear class variation (TW) in cranial measurements of *D. auricularis* with mean size differences for significantly different measurements expressed as a percentage (%). df = degrees of freedom, P = significance and *indicate significance at P<0.05

Levene's		GLS	IB	RH	RW	RL	PW	TBL	ML	MH	BM[1]	OH	UTR	LMC
sex	F	4.646788*	1.527784	2.937556	2.726805	3.874137*	1.062017	3.767987*	4.668608*	1.662136	1.261941	0.910091	1.29662	1.402304
	df	1	1	1	1	1	1	1	1	1	1	1	1	1
	P	0.011414*	0.221277	0.056883	0.069553	0.023465*	0.349039	0.025925	0.011186*	0.194144	0.286896	0.405294	0.277321	0.250099
TW	F	0.937542	0.516139	0.536337	1.043474	4.240897*	2.32206	0.28268	0.339869	1.307941	2.952206*	0.071845	2.35418	0.957927
	df	3	3	3	3	3	3	3	3	3	3	3	3	3
	P	0.424927	0.671962	0.658284	0.37607	0.006941*	0.078666	0.837817	0.796531	0.275141	0.035496*	0.974925	0.075552	0.415123
1-way ANOVA														
sex	S of S	5.712214	0.406053	0.181236	0.026017	0.82604	0.207253	1.88705	1.0729	0.006818	0.037018	0.995024	0.050291	0.057222
	F	0.905128	2.092298	0.692911	0.185715	0.811577	1.464004	1.574157	0.445079	0.029041	1.599492	2.122949	0.377454	0.396527
	df	1	1	1	1	1	1	1	1	1	1	1	1	1
	P	0.40728	0.127958	0.502142	0.830752	0.44662	0.235479	0.211502	0.641845	0.971383	0.206347	0.124227	0.686429	0.673545
TW	S of S	134.7124*	2.3888*	4.3605*	1.8306*	16.3423*	1.8742*	25.6584*	60.6837*	4.571*	0.0127	4.2051*	0.1336	0.1893
	df	3	3	3	3	3	3	3	3	3	3	3	3	3
	F	21.7695*	9.9131*	15.2333*	11.1398*	14.4335*	10.8863*	21.4931*	28.8694*	19.3558*	0.3556	6.5049*	0.6706	0.8813
	P	0*	0.000007*	0*	0.000002*	0*	0.000002*	0*	0*	0*	0.785199	0.000413*	0.57171	0.452992





Appendix 7: Phenogram generated from hierarchical cluster analysis using an unweighted pair group mean analysis (UPGMA) clustering algorithm of 11 individuals (6 females and 5 males) of *G. paeba* (A)6 individuals (3 males and female each) of *D. auricularis* (B).